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(54) Title: HUMAN ENDOTHELIN-BOMBESIN RECEPTOR

(57) Abstract

A human endothelin-bombesin receptor polypeptide and DNA (RNA) encoding such polypeptide and a procedure for producing such polypeptide by recombinant techniques is disclosed. Also disclosed are methods for utilizing such polypeptide for identifying agonists and antagonists to such polypeptide. Agonists to the endothelin-bombesin receptor polypeptide of the present invention may be used to treat asthma, Parkinson's Disease, acute heart failure, hypotension and osteoporosis. Antagonits against such polypeptides may be used therapeutically to treat hypertension, ulcerigenesis, subarachnoid hemorrhage, asthma, tumors, ciclosporing toxicity, cancer and septic shock.

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HUMAN ENDOTHELIN-BOMBESIN RECEPTOR

This invention relates to newly identified polynucleotides, polypeptides encoded by such polynucleotides, the use of such polynucleotides and polypeptides, as well as the production of such polynucleotides and polypeptides. More particularly, the polypeptide of the present invention is a human 7-transmembrane receptor. The transmembrane receptor is a G-protein coupled receptor. More particularly, the 7-transmembrane receptor has been putatively identified as an endothelin-bombesin receptor, sometimes hereinafter referred to as "ETBR." The invention also relates to inhibiting the action of such polypeptides.

It is well established that many medically significant biological processes are mediated by proteins participating in signal transduction pathways that involve G-proteins and/or second messengers, e.g., cAMP (Lefkowitz, Nature, 351:353-354 (1991)). Herein these proteins are referred to as proteins participating in pathways with G-proteins or PPG proteins. Some examples of these proteins include the GPC receptors, such as those for adrenergic agents and dopamine (Kobilka, B.K., et al., PNAS, 84:46-50 (1987); Kobilka, B.K., et al., Science, 238:650-656 (1987); Bunzow, J.R., et al., Nature, 336:783-787 (1988)), G-proteins themselves, effector

proteins, e.g., phospholipase C, adenyl cyclase, and phosphodiesterase, and actuator proteins, e.g., protein kinase A and protein kinase C (Simon, M.I., et al., Science, 252:802-8 (1991)).

For example, in one form of signal transduction, the effect of hormone binding is activation of an enzyme, adenylate cyclase, inside the cell. Enzyme activation by hormones is dependent on the presence of the nucleotide GTP, and GTP also influences hormone binding. A G-protein connects the hormone receptors to adenylate cyclase. G-protein was shown to exchange GTP for bound GDP when activated by hormone receptors. The GTP-carrying form then binds to an activated adenylate cyclase. Hydrolysis of GTP to GDP, catalyzed by the G-protein itself, returns the G-protein to its basal, inactive form. Thus, the G-protein serves a dual role, as an intermediate that relays the signal from receptor to effector, and as a clock that controls the duration of the signal.

The peptide endothelin is a peptide of 21 amino acid residues and performs *in vivo* effects via endothelin receptors. Endothelin (ET) is a peptide present in various tissues in animals and is known as a strong vasoconstrictor. ET is one peptide of a family of at least 4 mammalian peptides characterized by 2 disulphide bridges and 6 conserved amino acid residues at the C-terminus.

Members of the family are called endothelin-1 (ET-1), endothelin-2 (ET-2), and endothelin-3 (ET-3). A fourth peptide, vasointestinal contractor, is also sometimes described as the murine or rat form of ET-2. They differ mostly in the 29-membered ring system formed by the Cys-3-Cys-11 disulphide bond. Endothelins are produced by metabolism of a preproendothelin to a proendothelin, which is itself metabolized to the mature endothelin. The cleavage of proendothelin is thought to be due to the activity of a specific enzyme. ETs are distributed in a wide variety of

vascular and non-vascular tissues (PNAS, USA, 86:2863-2867 (1989)).

It has previously been shown *in vivo* that ET-1 and ET-2 are much stronger vasoconstrictors than ET-3, whereas the three ET isopeptides are roughly equipotent in producing the transient vasodilation. The analysis of nucleic acid sequences of ETs has revealed that various kinds of ET isopeptides exist. These ET isopeptides are also different in their properties. Therefore, it appears that various subtypes of ET-receptors exist. The existence of various subtypes of ET-receptors has been proven by the radioactive ligand binding studies of Watanabe, H., *et al.*, Biochem-Biophys, Res. Commun., 161:1252-1259 (1989), and Martin, E.R., *et al.*, J. Biol. Chem., 265:14044-14049 (1990). These studies indicate the existence of at least two kinds of ET-receptors. One of them has a higher affinity for ET-1 and ET-2 than for ET-3 and the other has an affinity for ET-1, ET-2 and ET-3 with no cell activity. The ET_A receptors have a lower affinity for ET-3 and the ET_B receptors are non-selective.

The receptors are homologous to other heptahelical receptors of the rhodopsin superfamily, having 7 hydrophobic regions predicted to form transmembrane helices.

The placenta has a very high expression of both receptors, as does the lung. In general the non-selective ET_B receptor seems to be more widely expressed (e.g., in liver, kidney and uterus) and is probably the more prominent receptor in the CNS, a result that agrees with binding and functional studies. The heart is the only tissue about which there is a consensus that an ET_A-type receptor predominates. The ET_A receptors are associated with blood vessels and ET_B receptors with glial, epithelial and ependymal cells, but few, if any, are associated with neurons. In the kidney, ET_A receptors are located on blood vessel smooth-muscle cells, and ET_B receptor expression occurs on a glomerular

endothelium, vasa recti and the thin segments of Henle's loops.

Endothelins elicit biological responses by various signal transduction mechanisms, including the G-protein-coupled activation of phospholipase C and the activation of voltage-dependent Ca^{2+} channels (Kasuya, Y., *et al.*, Biochem. Biophys. Res. Commun., 61:1049-1055 (1989)). Thus, different sub-types of the endothelin receptor may use different signal-transduction mechanisms. Endothelin receptors have a relatively long N terminus preceding transmembrane segment I, and this portion may be involved in binding a relatively large endothelin peptide.

Applicants have discovered a G-protein coupled receptor which has hydropathicity and amino acid homology which shows the existence of the 7 hydrophobic segments and a significant sequence similarity with other G-protein-coupled receptors. The 7 membrane-spanning domains and extra-cellular N-terminus and cytoplasmic C-terminus have also been identified.

The G-protein coupled receptor of the present invention has been putatively identified as an endothelin-bombesin receptor as a result of its homology to the known endothelin receptors ET_A and ET_B and as a result of its ability to bind endothelin and bombesin.

In accordance with one aspect of the present invention, there is provided a novel putative mature polypeptide which is a G-protein coupled receptor, as well as fragments, analogs and derivatives thereof. The polypeptide of the present invention is of human origin.

In accordance with another aspect of the present invention, there are provided polynucleotides (DNA or RNA) which encode such polypeptides.

In accordance with yet a further aspect of the present invention, there is provided a process for producing such polypeptides by recombinant techniques.

In accordance with yet a further aspect of the present invention, there is provided a process for utilizing such polypeptides, or polynucleotides encoding such polypeptides for therapeutic purposes, to measure the concentration of endothelin *in vivo*, or in soluble form as an antagonist.

In accordance with yet a further aspect of the present invention, there are provided antibodies against such polypeptides.

In accordance with yet another aspect of the present invention, there are provided antagonists to such polypeptides, which may be used to inhibit the action of such polypeptides.

In accordance with still another embodiment, there is provided a process for using the receptor to screen for receptor antagonists and/or receptor agonists and/or receptor ligands.

These and other aspects of the present invention should be apparent to those skilled in the art from the teachings herein.

The following drawings are illustrative of embodiments of the invention and are not meant to limit the scope of the invention as encompassed by the claims.

Figure 1 shows the cDNA sequence and the corresponding deduced amino acid sequence of the G-protein coupled receptor of the present invention. The first 26 amino acids represent a putative signal sequence. The standard one-letter abbreviation for amino acids is used.

Figure 2 is an illustration of the secondary structural features of the G-protein coupled receptor. The first 7 illustrations set forth the regions of the amino acid sequence which are alpha helices, beta sheets, turn regions or coiled regions. The boxed areas are the areas which correspond to the region indicated. The second set of figures illustrate areas of the amino acid sequence which are exposed to intracellular, cytoplasmic or are membrane-

spanning. The hydrophilicity part illustrates areas of the protein sequence which are the lipid bilay r f the membrane and are, therefore, hydrophobic, and areas outside the lipid bilayer membrane which are hydrophilic. The antigenic index corresponds to the hydrophilicity plot, since antigenic areas are areas outside the lipid bilayer membrane and are capable of binding antigens. The surface probability plot further corresponds to the antigenic index and the hydrophilicity plot. The amphipathic plots show those regions of the 13 sequences which are polar and non-polar. The flexible regions correspond to the second set of illustrations in the sense that flexible regions are those which are outside the membrane and inflexible regions are transmembrane regions.

Figure 3 illustrates an amino acid alignment of the G-protein coupled receptor of the present invention and endothelin receptors from various species of animals. Faded areas are those areas which match with the other amino acid sequences in the figure.

Figure 4 shows that ET1, ET3 and Bombesin induced chloride currents in oocytes injected with pHHPEC49 derived RNA transcripts. The trace shows ET1 mediated chloride current (nanoamps). Arrow indicates ET1 addition. The inset shows the mean peak responses to 10nM AII, Neuropeptide Y (NPY) and Bradykinin. The mean peak ± S.E. peak current response to ET1 is 150 ± 50 (n=75), ET2 156 ± 55 (n=75) and Bombesin 148 ± 47 (n=75).

It should be pointed out that sequencing inaccuracies are a common problem which occurs in polynucleotide sequences. Accordingly, the sequence of the drawing is based on several sequencing runs and the sequencing accuracy is considered to be at least 97%.

In accordance with an aspect of the present invention, there is provided an isolated nucleic acid (polynucleotide) which encodes for the mature polypeptide having the deduced amino acid sequence of Figure 1 or for the mature polypeptide

encoded by the cDNA of the clone deposited as ATCC Deposit No. 75823 on June 24, 1994.

A polynucleotide encoding a polypeptide of the present invention may be found in brain, liver and placenta. The polynucleotide of this invention was discovered in a cDNA library derived from a human brain. It is structurally related to the G protein-coupled receptor family. It contains an open reading frame encoding a protein of about 613 amino acid residues of which approximately the first 26 amino acids residues are the putative leader sequence such that the mature protein comprises 587 amino acids. The protein exhibits the highest degree of homology to a human ET_A receptor with 30 % identity and 55 % similarity over a 420 amino acid stretch.

The polynucleotide of the present invention may be in the form of RNA or in the form of DNA, which DNA includes cDNA, genomic DNA, and synthetic DNA. The DNA may be double-stranded or single-stranded, and if single stranded may be the coding strand or non-coding (anti-sense) strand. The coding sequence which encodes the mature polypeptide may be identical to the coding sequence shown in Figure 1 or that of the deposited clone or may be a different coding sequence which coding sequence, as a result of the redundancy or degeneracy of the genetic code, encodes the same mature polypeptide as the DNA of Figure 1 or the deposited cDNA.

The polynucleotide which encodes for the mature polypeptide of Figure 1 or for the mature polypeptide encoded by the deposited cDNA may include: only the coding sequence for the mature polypeptide; the coding sequence for the mature polypeptide and additional coding sequence such as a leader or secretory sequence or a proprotein sequence; the coding sequence for the mature polypeptide (and optionally additional coding sequence) and non-coding sequence, such as introns or non-coding sequence 5' and/or 3' of the coding sequence for the mature polypeptide.

Thus, the term "polynucleotide encoding a polypeptide" encompasses a polynucleotide which includes only coding sequence for the polypeptide as well as a polynucleotide which includes additional coding and/or non-coding sequence.

The present invention further relates to variants of the hereinabove described polynucleotides which encode for fragments, analogs and derivatives of the polypeptide having the deduced amino acid sequence of Figure 1 or the polypeptide encoded by the cDNA of the deposited clone. The variant of the polynucleotide may be a naturally occurring allelic variant of the polynucleotide or a non-naturally occurring variant of the polynucleotide.

Thus, the present invention includes polynucleotides encoding the same mature polypeptide as shown in Figure 1 or the same mature polypeptide encoded by the cDNA of the deposited clone as well as variants of such polynucleotides which variants encode for a fragment, derivative or analog of the polypeptide of Figure 1 or the polypeptide encoded by the cDNA of the deposited clone. Such nucleotide variants include deletion variants, substitution variants and addition or insertion variants.

As hereinabove indicated, the polynucleotide may have a coding sequence which is a naturally occurring allelic variant of the coding sequence shown in Figure 1 or of the coding sequence of the deposited clone. As known in the art, an allelic variant is an alternate form of a polynucleotide sequence which may have a substitution, deletion or addition of one or more nucleotides, which does not substantially alter the function of the encoded polypeptide.

The present invention also includes polynucleotides, wherein the coding sequence for the mature polypeptide may be fused in the same reading frame to a polynucleotide sequence which aids in expression and secretion of a polypeptide from a host cell, for example, a leader sequence which functions as a secretory sequence for controlling transport of a

polypeptide from the cell. The polypeptide having a leader sequenc is a preprotein and may have the leader sequence cleaved by the host cell to form the mature form f th polypeptide. The polynucleotides may also encode for a proprotein which is the mature protein plus additional 5' amino acid residues. A mature protein having a prosequence is a proprotein and is an inactive form of the protein. Once the prosequence is cleaved an active mature protein remains.

Thus, for example, the polynucleotide of the present invention may encode for a mature protein, or for a protein having a prosequence or for a protein having both a prosequence and a presequence (leader sequence).

The polynucleotides of the present invention may also have the coding sequence fused in frame to a marker sequence which allows for purification of the polypeptide of the present invention. The marker sequence may be a hexa-histidine tag supplied by a pQE-9 vector to provide for purification of the mature polypeptide fused to the marker in the case of a bacterial host, or, for example, the marker sequence may be a hemagglutinin (HA) tag when a mammalian host, e.g. COS-7 cells, is used. The HA tag corresponds to an epitope derived from the influenza hemagglutinin protein (Wilson, I., et al., Cell, 37:767 (1984)).

The present invention further relates to polynucleotides which hybridize to the hereinabove-described sequences if there is at least 50% and preferably 70% identity between the sequences. The present invention particularly relates to polynucleotides which hybridize under stringent conditions to the hereinabove-described polynucleotides . As herein used, the term "stringent conditions" means hybridization will occur only if there is at least 95% and preferably at least 97% identity between the sequences. The polynucleotides which hybridize to the hereinabove described polynucleotides in a preferred embodiment encode polypeptides which either retain

substantially the same biological function or activity as the mature polypeptide encoded by the cDNA of Figure 1 or the deposited cDNA, i.e. function as a G-protein coupled receptor or retain the ability to bind the ligand for the receptor even though the polypeptide does not function as a G-protein coupled receptor, for example, a soluble form of the receptor.

The deposit(s) referred to herein will be maintained under the terms of the Budapest Treaty on the International Recognition of the Deposit of Micro-organisms for purposes of Patent Procedure. These deposits are provided merely as convenience to those of skill in the art and are not an admission that a deposit is required under 35 U.S.C. §112. The sequence of the polynucleotides contained in the deposited materials, as well as the amino acid sequence of the polypeptides encoded thereby, are incorporated herein by reference and are controlling in the event of any conflict with any description of sequences herein. A license may be required to make, use or sell the deposited materials, and no such license is hereby granted.

The present invention further relates to a G-protein coupled receptor polypeptide which has the deduced amino acid sequence of Figure 1 or which has the amino acid sequence encoded by the deposited cDNA, as well as fragments, analogs and derivatives of such polypeptide.

The terms "fragment," "derivative" and "analog" when referring to the polypeptide of Figure 1 or that encoded by the deposited cDNA, means a polypeptide which either retains substantially the same biological function or activity as such polypeptide, i.e. functions as a G-protein coupled receptor, or retains the ability to bind the ligand or the receptor even though the polypeptide does not function as a G-protein coupled receptor, for example, a soluble form of the receptor. An analog includes a proprotein which can be

activated by cleavage of the proprotein portion to produc an active mature polypeptid .

The polypeptide of the present invention may be a recombinant polypeptide, a natural polypeptide or a synthetic polypeptide, preferably a recombinant polypeptide.

The fragment, derivative or analog of the polypeptide of Figure 1 or that encoded by the deposited cDNA may be (i) one in which one or more of the amino acid residues are substituted with a conserved or non-conserved amino acid residue (preferably a conserved amino acid residue) and such substituted amino acid residue may or may not be one encoded by the genetic code, or (ii) one in which one or more of the amino acid residues includes a substituent group, or (iii) one in which the mature polypeptide is fused with another compound, such as a compound to increase the half-life of the polypeptide (for example, polyethylene glycol), or (iv) one in which the additional amino acids are fused to the mature polypeptide, such as a leader or secretory sequence or a sequence which is employed for purification of the mature polypeptide or a proprotein sequence. Such fragments, derivatives and analogs are deemed to be within the scope of those skilled in the art from the teachings herein.

The polypeptides and polynucleotides of the present invention are preferably provided in an isolated form, and preferably are purified to homogeneity.

The term "isolated" means that the material is removed from its original environment (e.g., the natural environment if it is naturally occurring). For example, a naturally-occurring polynucleotide or polypeptide present in a living animal is not isolated, but the same polynucleotide or polypeptide, separated from some or all of the coexisting materials in the natural system, is isolated. Such polynucleotides could be part of a vector and/or such polynucleotides or polypeptides could be part of a

composition, and still be isolated in that such vector or composition is not part of its natural environment.

The present invention also relates to vectors which include polynucleotides of the present invention, host cells which are genetically engineered with vectors of the invention and the production of polypeptides of the invention by recombinant techniques.

Host cells are genetically engineered (transduced or transformed or transfected) with the vectors of this invention which may be, for example, a cloning vector or an expression vector. The vector may be, for example, in the form of a plasmid, a viral particle, a phage, etc. The engineered host cells can be cultured in conventional nutrient media modified as appropriate for activating promoters, selecting transformants or amplifying the ETBR genes. The culture conditions, such as temperature, pH and the like, are those previously used with the host cell selected for expression, and will be apparent to the ordinarily skilled artisan.

The polynucleotides of the present invention may be employed for producing polypeptides by recombinant techniques. Thus, for example, the polynucleotide may be included in any one of a variety of expression vectors for expressing a polypeptide. Such vectors include chromosomal, nonchromosomal and synthetic DNA sequences, e.g., derivatives of SV40; bacterial plasmids; phage DNA; baculovirus; yeast plasmids; vectors derived from combinations of plasmids and phage DNA, viral DNA such as vaccinia, adenovirus, fowl pox virus, and pseudorabies. However, any other vector may be used as long as it is replicable and viable in the host.

The appropriate DNA sequence may be inserted into the vector by a variety of procedures. In general, the DNA sequence is inserted into an appropriate restriction endonuclease site(s) by procedures known in the art. Such

procedures and others are deemed to be within the scope of those skilled in the art.

The DNA sequence in the expression vector is operatively linked to an appropriate expression control sequence(s) (promoter) to direct mRNA synthesis. As representative examples of such promoters, there may be mentioned: LTR or SV40 promoter, the E. coli. lac or trp, the phage lambda P_L promoter and other promoters known to control expression of genes in prokaryotic or eukaryotic cells or their viruses. The expression vector also contains a ribosome binding site for translation initiation and a transcription terminator. The vector may also include appropriate sequences for amplifying expression.

In addition, the expression vectors preferably contain one or more selectable marker genes to provide a phenotypic trait for selection of transformed host cells such as dihydrofolate reductase or neomycin resistance for eukaryotic cell culture, or such as tetracycline or ampicillin resistance in E. coli.

The vector containing the appropriate DNA sequence as hereinabove described, as well as an appropriate promoter or control sequence, may be employed to transform an appropriate host to permit the host to express the protein.

As representative examples of appropriate hosts, there may be mentioned: bacterial cells, such as E. coli, Streptomyces, Salmonella typhimurium; fungal cells, such as yeast; insect cells such as Drosophila and Sf9; animal cells such as CHO, COS or Bowes melanoma; plant cells, etc. The selection of an appropriate host is deemed to be within the scope of those skilled in the art from the teachings herein.

More particularly, the present invention also includes recombinant constructs comprising one or more of the sequences as broadly described above. The constructs comprise a vector, such as a plasmid or viral vector, into which a sequence of the invention has been inserted, in a

forward or reverse orientation. In a preferred aspect of this embodiment, the construct further comprises regulatory s quences, including, for example, a promoter, operably linked to the sequence. Large numbers of suitable vectors and promoters are known to those of skill in the art, and are commercially available. The following vectors are provided by way of example. Bacterial: pQE70, pQE60, pQE-9 (Qiagen), pbs, pD10, phagescript, psiX174, pbluescript SK, pbsks, pNH8A, pNH16a, pNH18A, pNH46A (Stratagene); ptrc99a, pKK223-3, pKK233-3, pDR540, pRIT5 (Pharmacia). Eukaryotic: pWLNEO, pSV2CAT, pOG44, pXT1, pSG (Stratagene) pSVK3, pBPV, pMSG, pSVL (Pharmacia). However, any other plasmid or vector may be used as long as they are replicable and viable in the host.

Promoter regions can be selected from any desired gene using CAT (chloramphenicol transferase) vectors or other vectors with selectable markers. Two appropriate vectors are PKK232-8 and PCM7. Particular named bacterial promoters include lacI, lacZ, T3, T7, gpt, lambda P_R, P_L and trp. Eukaryotic promoters include CMV immediate early, HSV thymidine kinase, early and late SV40, LTRs from retrovirus, and mouse metallothionein-I. Selection of the appropriate vector and promoter is well within the level of ordinary skill in the art.

In a further embodiment, the present invention relates to host cells containing the above-described constructs. The host cell can be a higher eukaryotic cell, such as a mammalian cell, or a lower eukaryotic cell, such as a yeast cell, or the host cell can be a prokaryotic cell, such as a bacterial cell. Introduction of the construct into the host cell can be effected by calcium phosphate transfection, DEAE-Dextran mediated transfection, or electroporation. (Davis, L., Dibner, M., Battey, I., Basic Methods in Molecular Biology, (1986)).

The constructs in host cells can b used in a conventional manner to produce the gene product encod d by the recombinant sequence. Alternatively, the polypeptides f the invention can be synthetically produced by conventional peptide synthesizers.

Mature proteins can be expressed in mammalian cells, yeast, bacteria, or other cells under the control of appropriate promoters. Cell-free translation systems can also be employed to produce such proteins using RNAs derived from the DNA constructs of the present invention. Appropriate cloning and expression vectors for use with prokaryotic and eukaryotic hosts are described by Sambrook, et al., Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor, N.Y., (1989), the disclosure of which is hereby incorporated by reference.

Transcription of the DNA encoding the polypeptides of the present invention by higher eukaryotes is increased by inserting an enhancer sequence into the vector. Enhancers are cis-acting elements of DNA, usually about from 10 to 300 bp that act on a promoter to increase its transcription. Examples including the SV40 enhancer on the late side of the replication origin bp 100 to 270, a cytomegalovirus early promoter enhancer, the polyoma enhancer on the late side of the replication origin, and adenovirus enhancers.

Generally, recombinant expression vectors will include origins of replication and selectable markers permitting transformation of the host cell, e.g., the ampicillin resistance gene of E. coli and S. cerevisiae TRP1 gene, and a promoter derived from a highly-expressed gene to direct transcription of a downstream structural sequence. Such promoters can be derived from operons encoding glycolytic enzymes such as 3-phosphoglycerate kinase (PGK), α-factor, acid phosphatase, or heat shock proteins, among others. The heterologous structural sequence is assembled in appropriate phase with translation initiation and termination sequences,

and preferably, a leader sequence capabl of directing secretion of translated protein into the periplasmic space or extracellular medium. Optionally, th heterologous sequ nce can encode a fusion protein including an N-terminal identification peptide imparting desired characteristics, e.g., stabilization or simplified purification of expressed recombinant product.

Useful expression vectors for bacterial use are constructed by inserting a structural DNA sequence encoding a desired protein together with suitable translation initiation and termination signals in operable reading phase with a functional promoter. The vector will comprise one or more phenotypic selectable markers and an origin of replication to ensure maintenance of the vector and to, if desirable, provide amplification within the host. Suitable prokaryotic hosts for transformation include E. coli, Bacillus subtilis, Salmonella typhimurium and various species within the genera Pseudomonas, Streptomyces, and Staphylococcus, although others may also be employed as a matter of choice.

As a representative but nonlimiting example, useful expression vectors for bacterial use can comprise a selectable marker and bacterial origin of replication derived from commercially available plasmids comprising genetic elements of the well known cloning vector pBR322 (ATCC 37017). Such commercial vectors include, for example, pKK223-3 (Pharmacia Fine Chemicals, Uppsala, Sweden) and GEM1 (Promega Biotec, Madison, WI, USA). These pBR322 "backbone" sections are combined with an appropriate promoter and the structural sequence to be expressed.

Following transformation of a suitable host strain and growth of the host strain to an appropriate cell density, the selected promoter is induced by appropriate means (e.g., temperature shift or chemical induction) and cells are cultured for an additional period.

Cells are typically harvested by centrifugation, disrupted by physical or chemical means, and the resulting crude extract retained f r further purificati n.

Microbial cells employed in expression of proteins can be disrupted by any convenient method, including freeze-thaw cycling, sonication, mechanical disruption, or use of cell lysing agents, such methods are well know to those skilled in the art.

Various mammalian cell culture systems can also be employed to express recombinant protein. Examples of mammalian expression systems include the COS-7 lines of monkey kidney fibroblasts, described by Gluzman, Cell, 23:175 (1981), and other cell lines capable of expressing a compatible vector, for example, the C127, 3T3, CHO, HeLa and BHK cell lines. Mammalian expression vectors will comprise an origin of replication, a suitable promoter and enhancer, and also any necessary ribosome binding sites, polyadenylation site, splice donor and acceptor sites, transcriptional ~~termination~~ sequences, and 5' flanking nontranscribed sequences. DNA sequences derived from the SV40 splice, and polyadenylation sites may be used to provide the required nontranscribed genetic elements.

The G-protein coupled receptor polypeptides can be recovered and purified from recombinant cell cultures by methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography hydroxylapatite chromatography and lectin chromatography. Protein refolding steps can be used, as necessary, in completing configuration of the mature protein. Finally, high performance liquid chromatography (HPLC) can be employed for final purification steps.

The polypeptides of the present invention may be a naturally purified product, or a product of chemical

synthetic procedures, or produced by recombinant techniques from a prokaryotic or eukaryotic host (f r example, by bacterial, yeast, higher plant, insect and mammalian cells in culture). Depending upon the host employed in a recombinant production procedure, the polypeptides of the present invention may be glycosylated or may be non-glycosylated. Polypeptides of the invention may also include an initial methionine amino acid residue.

Bombesin, in addition to endothelin, has been found to bind to and stimulate the receptor of the present invention. Bombesin is a tetradecapeptide which has as a mammalian homolog the 27-amino acid peptide gastrin-releasing peptide (GRP). Bombesin is regarded as one of the most potent peptide to affect the central nervous system, since it has been reported as a thermoregulator in the rat (Brown, M. et al., Science, 196:998-1000 (1977)). Also, bombesin/gastrin releasing peptide is synthesized and secreted by small cell lung cancers (Davis, T.P. et al., Peptides, 13:401-17 (1992)).

The G-protein coupled receptor of the present invention may be employed in a process for screening for agonists and/or antagonists for the receptor.

In general, such screening procedures involve providing appropriate cells which express the receptor on the surface thereof. In particular, a polynucleotide encoding the receptor of the present invention is employed to transfect cells to thereby express the G-protein coupled receptor. Such transfection may be accomplished by procedures as hereinabove described.

One such screening procedure involves the use of a melanophore which are transfected to express the G-protein coupled receptor of the present invention. Such a screening technique is described in PCT WO 92/01810 published February 6, 1992.

Thus, for example, such assay may b employed for screening for a receptor antagonist by contacting the melanophore cells which encode th G-protein coupled rec ptor with both the receptor ligand and a compound to be screened. Inhibition of the signal generated by the ligand indicates that a compound is a potential antagonist for the receptor, i.e., inhibits activation of the receptor.

The screen may be employed for determining an agonist by contacting such cells with compounds to be screened and determining whether such compound generates a signal, i.e., activates the receptor.

Other screening techniques include the use of cells which express the G-protein coupled receptor (for example, transfected CHO cells) in a system which measures extracellular pH changes caused by receptor activation, for example, as described in Science, volume 246, pages 181-296 (October 1989). For example, potential agonists or antagonists may be contacted with a cell which expresses the G-protein coupled receptor and a second messenger response, e.g. signal transduction or pH changes, may be measured to determine whether the potential agonist or antagonist is effective.

Another such screening technique involves introducing RNA encoding the G-protein coupled receptor into Xenopus oocytes to transiently express the receptor. The receptor oocytes may then be contacted with the receptor ligand and a compound to be screened, followed by detection of inhibition of a calcium signal.

Another screening technique involves expressing the G-protein coupled receptor in which the receptor is linked to a phospholipase C or D. As representative examples of such cells, there may be mentioned endothelial cells, smooth muscle cells, embryonic kidney cells, etc. The screening for an antagonist or agonist may be accomplished as hereinabove described by detecting activation of the receptor or

inhibition of activation of the receptor from the phospholipase second signal.

Another method involves screening for antagonists by determining inhibition of binding of labeled ligand to cells which have the receptor on the surface thereof. Such a method involves transfecting a eukaryotic cell with DNA encoding the G-protein coupled receptor such that the cell expresses the receptor on its surface and contacting the cell with a potential antagonist in the presence of a labeled form of a known ligand. The ligand can be labeled, e.g., by radioactivity. The amount of labeled ligand bound to the receptors is measured, e.g., by measuring radioactivity of the receptors. If the potential antagonist binds to the receptor as determined by a reduction of labeled ligand which binds to the receptors, the binding of labeled ligand to the receptor is inhibited.

In general, antagonists for G-protein coupled receptors which are determined by such screening procedures may be employed for a variety of therapeutic purposes. For example, such antagonists have been employed for treatment of hypertension, angina pectoris, myocardial infarction, ulcers, asthma, allergies, psychoses, depression, migraine, vomiting, and benign prostatic hypertrophy.

Agonists for G-protein coupled receptors are also useful for therapeutic purposes, such as the treatment of asthma, Parkinson's disease, acute heart failure, hypotension, urinary retention, and osteoporosis.

Examples of potential antagonists include an antibody, or in some cases an oligonucleotide, which binds to the G-protein coupled receptor but does not elicit a second messenger response such that the activity of the G-protein coupled receptor is prevented. Potential antagonists also include proteins which are closely related to the ligand of the G-protein coupled receptor, i.e. a fragment of the

ligand, which hav l st biological functi n and when binding to the G-protein coupled receptor, elicit no response.

A potential antagonist also includes an antisense construct prepared through the use of antisense technology. Antisense technology can be used to control gene expression through triple-helix formation or antisense DNA or RNA, both of which methods are based on binding of a polynucleotide to DNA or RNA. For example, the 5' coding portion of the polynucleotide sequence, which encodes for the mature polypeptides of the present invention, is used to design an antisense RNA oligonucleotide of from about 10 to 40 base pairs in length. A DNA oligonucleotide is designed to be complementary to a region of the gene involved in transcription (triple helix -see Lee et al., Nucl. Acids Res., 6:3073 (1979); Cooney et al, Science, 241:456 (1988); and Dervan et al., Science, 251: 1360 (1991)), thereby preventing transcription and the production of G-protein coupled receptor. The antisense RNA oligonucleotide hybridizes to the mRNA *in vivo* and blocks translation of the mRNA molecule into the G-protein coupled receptor (antisense - Okano, J. Neurochem., 56:560 (1991); Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988)). The oligonucleotides described above can also be delivered to cells such that the antisense RNA or DNA may be expressed *in vivo* to inhibit production of G-protein coupled receptor.

Another potential antagonist is a small molecule which binds to the G-protein coupled receptor, making it inaccessible to ligands such that normal biological activity is prevented. Examples of small molecules include but are not limited to small peptides or peptide-like molecules.

Potential antagonists also include a soluble G-protein coupled receptor, e.g. a fragment of the receptor, which binds to the ligand and prevents the ligand from interacting with membrane bound G-protein coupled receptors.

An endothelin antagonist may be employed to offset the vasoconstrictive effects of endothelin and, th refor , may be employed t treat hypertension through vasodilation. These antagonists may also be used to treat the long-lasting vasospasms due to subarachnoid hemorrhages which cause increases in endothelin levels in cerebrospinal fluid and plasma.

Endothelin antagonist may also be used to treat ulcerogenesis and gastric lesions. ET-1 and ET-3 induce gastric lesions and enhance alcohol-induced lesions. Accordingly, inhibiting ET-1 and ET-3 from interacting with the ETBRs can prevent these conditions.

Endothelins potently contract pulmonary smooth muscle and levels of endothelins are increased in pulmonary lavage fluid during asthmatic attacks, therefore, antagonists for diminishing or preventing binding of endothelin may be used to treat asthma.

Endothelin levels are increased in cancer tissue and a cancer-derived cell line can be stimulated to produce endothelin. ET-1 itself stimulates growth of cancerous cells. Accordingly, endothelin antagonists may be employed to prevent the growth of cancer cells and tumors.

An increase in circulating endothelin levels is increased by ciclosporin, which may explain the toxic effects of ciclosporin. Accordingly, endothelin antagonists may be used to prevent and/or treat ciclosporin toxicity.

Endothelin antagonists may also be used to treat septic shock which is caused by pathological levels of endothelins. Further, hypertension, congestive heart failure, coronary artery disease, atherosclerosis, restenosis, benign prostatic hypertrophy, renal failure and stroke may also be treated with the antagonist of ETBRs.

Bombesin antagonists may be employed to treat small cell lung cancers which synthesize and secret bombesin/gastrin releasing peptide. A bombesin antagonist will prevent

bombesin from stimulating the ETBR of the present invention.

The antagonists may be employed in a composition with a pharmaceutically acceptable carrier, e.g., as hereinafter described.

The present invention also provides a method for determining whether a ligand not known to be capable of binding to a G-protein coupled receptor can bind to such receptor which comprises contacting a mammalian cell which expresses a G-protein coupled receptor with the ligand under conditions permitting binding of ligands to the G-protein coupled receptor, detecting the presence of a ligand which binds to the receptor and thereby determining whether the ligand binds to the G-protein coupled receptor. The systems hereinabove described for determining agonists and/or antagonists may also be employed for determining ligands which bind to the receptor.

The ETBR polypeptides and antagonists or agonists which are polypeptides, may be employed in accordance with the present invention by expression of such polypeptides *in vivo*, which is often referred to as "gene therapy."

Thus, for example, cells from a patient may be engineered with a polynucleotide (DNA or RNA) encoding a polypeptide *ex vivo*, with the engineered cells then being provided to a patient to be treated with the polypeptide. Such methods are well-known in the art. For example, cells may be engineered by procedures known in the art by use of a retroviral particle containing RNA encoding a polypeptide of the present invention.

Similarly, cells may be engineered *in vivo* for expression of a polypeptide *in vivo* by, for example, procedures known in the art. As known in the art, a producer cell for producing a retroviral particle containing RNA encoding the polypeptide of the present invention may be administered to a patient for engineering cells *in vivo* and expression of the polypeptide *in vivo*. These and other

methods for administering a polypeptide of the present invention by such method should be apparent to those skilled in the art from the teachings of the present invention. For example, the expression vehicle for engineering cells may be other than a retrovirus, for example, an adenovirus which may be used to engineer cells in vivo after combination with a suitable delivery vehicle.

The ETBR polypeptides and antagonists or agonists may be employed in combination with a suitable pharmaceutical carrier. Such compositions comprise a therapeutically effective amount of the polypeptide, and a pharmaceutically acceptable carrier or excipient. Such a carrier includes but is not limited to saline, buffered saline, dextrose, water, glycerol, ethanol, and combinations thereof. The formulation should suit the mode of administration.

The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions of the invention. Associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration. In addition, the polypeptides and agonists and antagonists may be employed in conjunction with other therapeutic compounds.

The pharmaceutical compositions may be administered in a convenient manner such as by the topical, intravenous, intraperitoneal, intramuscular, subcutaneous, intranasal or intradermal routes. The pharmaceutical compositions are administered in an amount which is effective for treating and/or prophylaxis of the specific indication. In general, the pharmaceutical compositions will be administered in an amount of at least about 10 μg/kg body weight and in most cases they will be administered in an amount not in excess of about 8 mg/Kg body weight per day. In most cases, the dosage

is from about 10 μg/kg to about 1 mg/kg body w ight daily, taking into account the rout s of administration, symptoms, etc.

According to a further aspect of the present invention, a soluble form of the endothelin receptor of the present invention may be used as part of a diagnostic assay to detect levels of endothelin *in vivo*. An example of such an assay comprises removing a sample from a patient and incubating the endothelin receptors with the sample, isolating the endothelin receptors and determining the percent endothelin receptors which have interacted with and binded to endothelin. An altered level of endothelin is indicative of certain disorders or diseases.

The sequences of the present invention are also valuable for chromosome identification. The sequence is specifically targeted to and can hybridize with a particular location on an individual human chromosome. Moreover, there is a current need for identifying particular sites on the chromosome. Few chromosome marking reagents based on actual sequence data (repeat polymorphisms) are presently available for marking chromosomal location. The mapping of DNAs to chromosomes according to the present invention is an important first step in correlating those sequences with genes associated with disease.

Briefly, sequences can be mapped to chromosomes by preparing PCR primers (preferably 15-25 bp) from the cDNA. Computer analysis of the cDNA is used to rapidly select primers that do not span more than one exon in the genomic DNA, thus complicating the amplification process. These primers are then used for PCR screening of somatic cell hybrids containing individual human chromosomes. Only those hybrids containing the human gene corresponding to the primer will yield an amplified fragment.

PCR mapping of somatic cell hybrids is a rapid procedure for assigning a particular DNA to a particular chromosome.

Using the present invention with the same oligonucleotide primers, sublocalization can be achieved with panels of fragments from specific chromosomes or pools of large genomic clones in an analogous manner. Other mapping strategies that can similarly be used to map to its chromosome include *in situ* hybridization, prescreening with labeled flow-sorted chromosomes and preselection by hybridization to construct chromosome specific-cDNA libraries.

Fluorescence *in situ* hybridization (FISH) of a cDNA clone to a metaphase chromosomal spread can be used to provide a precise chromosomal location in one step. This technique can be used with cDNA as short as 500 or 600 bases; however, clones larger than 2,000 bp have a higher likelihood of binding to a unique chromosomal location with sufficient signal intensity for simple detection. FISH requires use of the clones from which the EST was derived, and the longer the better. For example, 2,000 bp is good, 4,000 is better, and more than 4,000 is probably not necessary to get good results a reasonable percentage of the time. For a review of this technique, see Verma et al., Human Chromosomes: a Manual of Basic Techniques, Pergamon Press, New York (1988).

Once a sequence has been mapped to a precise chromosomal location, the physical position of the sequence on the chromosome can be correlated with genetic map data. Such data are found, for example, in V. McKusick, Mendelian Inheritance in Man (available on line through Johns Hopkins University Welch Medical Library). The relationship between genes and diseases that have been mapped to the same chromosomal region are then identified through linkage analysis (coinheritance of physically adjacent genes).

Next, it is necessary to determine the differences in the cDNA or genomic sequence between affected and unaffected individuals. If a mutation is observed in some or all of the affected individuals but not in any normal individuals, then

the mutation is likely to be the causative agent of the diseas .

With current resolution of physical mapping and gen tic mapping techniques, a cDNA precisely localized to a chromosomal region associated with the disease could be one of between 50 and 500 potential causative genes. (This assumes 1 megabase mapping resolution and one gene per 20 kb).

The polypeptides, their fragments or other derivatives, or analogs thereof, or cells expressing them can be used as an immunogen to produce antibodies thereto. These antibodies can be, for example, polyclonal or monoclonal antibodies. The present invention also includes chimeric, single chain, and humanized antibodies, as well as Fab fragments, or the product of an Fab expression library. Various procedures known in the art may be used for the production of such antibodies and fragments.

Antibodies generated against the polypeptides corresponding to a sequence of the present invention can be obtained by direct injection of the polypeptides into an animal or by administering the polypeptides to an animal, preferably a nonhuman. The antibody so obtained will then bind the polypeptides itself. In this manner, even a sequence encoding only a fragment of the polypeptides can be used to generate antibodies binding the whole native polypeptides. Such antibodies can then be used to isolate the polypeptide from tissue expressing that polypeptide.

For preparation of monoclonal antibodies, any technique which provides antibodies produced by continuous cell line cultures can be used. Examples include the hybridoma technique (Kohler and Milstein, 1975, Nature, 256:495-497), the trioma technique, the human B-cell hybridoma technique (Kozbor et al., 1983, Immunology Today 4:72), and the EBV-hybridoma technique to produce human monoclonal antibodies

(Cole, et al., 1985, in Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, Inc., pp. 77-96).

Techniques described for the production of single chain antibodies (U.S. Patent 4,946,778) can be adapted to produce single chain antibodies to immunogenic polypeptide products of this invention.

The present invention will be further described with reference to the following examples; however, it is to be understood that the present invention is not limited to such examples. All parts or amounts, unless otherwise specified, are by weight.

In order to facilitate understanding of the following examples certain frequently occurring methods and/or terms will be described.

"Plasmids" are designated by a lower case p preceded and/or followed by capital letters and/or numbers. The starting plasmids herein are either commercially available, publicly available on an unrestricted basis, or can be constructed from available plasmids in accord with published procedures. In addition, equivalent plasmids to those described are known in the art and will be apparent to the ordinarily skilled artisan.

"Digestion" of DNA refers to catalytic cleavage of the DNA with a restriction enzyme that acts only at certain sequences in the DNA. The various restriction enzymes used herein are commercially available and their reaction conditions, cofactors and other requirements were used as would be known to the ordinarily skilled artisan. For analytical purposes, typically 1 μg of plasmid or DNA fragment is used with about 2 units of enzyme in about 20 μl of buffer solution. For the purpose of isolating DNA fragments for plasmid construction, typically 5 to 50 μg of DNA are digested with 20 to 250 units of enzyme in a larger volume. Appropriate buffers and substrate amounts for particular restriction enzymes are specified by the

manufacturer. Incubation times of about 1 hour at 37°C are ordinarily used, but may vary in accordance with th supplier's instructions. After digesti n th reaction is electrophoresed directly on a polyacrylamide gel to isolate the desired fragment.

Size separation of the cleaved fragments is performed using 8 percent polyacrylamide gel described by Goeddel, D. et al., Nucleic Acids Res., 8:4057 (1980).

"Oligonucleotides" refers to either a single stranded polydeoxynucleotide or two complementary polydeoxynucleotide strands which may be chemically synthesized. Such synthetic oligonucleotides have no 5' phosphate and thus will not ligate to another oligonucleotide without adding a phosphate with an ATP in the presence of a kinase. A synthetic oligonucleotide will ligate to a fragment that has not been dephosphorylated.

"Ligation" refers to the process of forming phosphodiester bonds between two double stranded nucleic acid fragments (Maniatis, T., et al., Id., p. 146). Unless otherwise provided, ligation may be accomplished using known buffers and conditions with 10 units to T4 DNA ligase ("ligase") per 0.5 μg of approximately equimolar amounts of the DNA fragments to be ligated.

Unless otherwise stated, transformation was performed as described in the method of Graham, F. and Van der Eb, A., Virology, 52:456-457 (1973).

Example 1

Bacterial Expression and Purification of ETBR

The DNA sequence encoding for ETBR, ATCC # 75823, is initially amplified using PCR oligonucleotide primers corresponding to the 5' and sequences of the processed ETBR protein (minus the signal peptide sequence) and the vector sequences 3' to the ETBR gene. Additional nucleotides corresponding to ETBR were added to the 5' and 3' sequences

respectively. The 5' oligonucleotide primer has the sequence 5' CACTAAGCTTAATGCGAGCCCCGGGCGCG 3' contains a HindIII restriction enzyme site followed by 18 nucleotides f ETBR coding sequence starting from the presumed terminal amino acid of the processed protein codon. The 3' sequence 5' GAACTTCTAGACCGTCAGCAATGAGTACCGAC 3' contains complementary sequences to an XbaI site and is followed by 18 nucleotides of ETBR. The restriction enzyme sites correspond to the restriction enzyme sites on the bacterial expression vector pQE-9 (Qiagen, Inc. 9259 Eton Avenue, Chatsworth, CA, 91311). pQE-9 encodes antibiotic resistance (Amp^r), a bacterial origin of replication (ori), an IPTG-regulatable promoter operator (P/O), a ribosome binding site (RBS), a 6-His tag and restriction enzyme sites. pQE-9 was then digested with HindIII and XbaI. The amplified sequences were ligated into pQE-9 and were inserted in frame with the sequence encoding for the histidine tag and the RBS. The ligation mixture was then used to transform E. coli HB101 by the procedure described in Sambrook, J. et al., Molecular Cloning: A Laboratory Manual, Cold Spring Laboratory Press, (1989). Transformants are identified by their ability to grow on LB plates and ampicillin/kanamycin resistant colonies were selected. Plasmid DNA was isolated and confirmed by restriction analysis. Clones containing the desired constructs were grown overnight (O/N) in liquid culture in LB media supplemented with both Amp (100 ug/ml) and Kan (25 ug/ml). The O/N culture is used to inoculate a large culture at a ratio of 1:100 to 1:250. The cells were grown to an optical density 600 ($O.D.^{600}$) of between 0.4 and 0.6. IPTG ("Isopropyl-B-D-thiogalacto pyranoside") was then added to a final concentration of 1 mM. IPTG induces by inactivating the lacI repressor, clearing the P/O leading to increased gene expression. Cells were grown an extra 3 to 4 hours. Cells were then harvested by centrifugation. The cell pellet was solubilized in the chaotropic agent 6 Molar Guanidine

HCl. After clarification, solubilized ETBR was purified from this solution by chromatography on a Nickel-Chelat column under conditions that allow for tight binding by proteins containing the 6-His tag (Hochuli, E. et al., J. Chromatography 411:177-184 (1984)). ETBR was eluted from the column in 6 molar guanidine HCl pH 5.0 and for the purpose of renaturation adjusted to 3 molar guanidine HCl, 100mM sodium phosphate, 10 mmolar glutathione (reduced) and 2 mmolar glutathione (oxidized). After incubation in this solution for 12 hours the protein was dialyzed to 10 mmolar sodium phosphate.

Example 2

Expression of Recombinant ETBR in COS cells

The expression of plasmid, pETBR HA is derived from a vector pcDNAI/Amp (Invitrogen) containing: 1) SV40 origin of replication, 2) ampicillin resistance gene, 3) E.coli replication origin, 4) CMV promoter followed by a polylinker region, a SV40 intron and polyadenylation site. A DNA fragment encoding the entire ETBR protein and a HA tag fused in frame to its 3' end was cloned into the polylinker region of the vector, therefore, the recombinant protein expression is directed under the CMV promoter. The HA tag correspond to an epitope derived from the influenza hemagglutinin protein as previously described (I. Wilson, H. Niman, R. Heighten, A Cherenson, M. Connolly, and R. Lerner, 1984, Cell 37, 767). The infusion of HA tag to our target protein allows easy detection of the recombinant protein with an antibody that recognizes the HA epitope.

The plasmid construction strategy is described as follows:

The DNA sequence encoding for ETBR, ATCC # 75823, was constructed by PCR on the original EST cloned using two primers: the 5' primer 5' GTCCAAGCTTGCCACCATGCGAGCCCCGGGCGCG 3' contains a HindIII site followed by 18 nucleotides of ETBR

coding sequence starting from the initiation cod n; the 3' sequence 5' CTAGCTCGAGTCAAGCGTAGTCTGGGACGTCGTATGGGTAGCAGCAAT GAGTTCCGACAGA 3' contains complementary s quences to an XhoI site, translation stop codon, HA tag and the last 18 nucleotides of the ETBR coding sequence (not including the stop codon). Therefore, the PCR product contains a HindIII site, ETBR coding sequence followed by HA tag fused in frame, a translation termination stop codon next to the HA tag, and an XhoI site. The PCR amplified DNA fragment and the vector, pcDNAI/Amp, were digested with HindIII and XhoI restriction enzyme and ligated. The ligation mixture was transformed into E. coli strain SURE (available from Stratagene Cloning Systems, 11099 North Torrey Pines Road, La Jolla, CA 92037) the transformed culture was plated on ampicillin media plates and resistant colonies were selected. Plasmid DNA was isolated from transformants and examined by restriction analysis for the presence of the correct fragment. For expression of the recombinant ETBR, COS cells were transfected with the expression vector by DEAE-DEXTRAN method (J. Sambrook, E. Fritsch, T. Maniatis, Molecular Cloning: A Laboratory Manual, Cold Spring Laboratory Press, (1989)). The expression of the ETBR HA protein was detected by radiolabelling and immunoprecipitation method (E. Harlow, D. Lane, Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, (1988)). Cells were labelled for 8 hours with ^{35}S-cysteine two days post transfection. Culture media were then collected and cells were lysed with detergent (RIPA buffer (150 mM NaCl, 1% NP-40, 0.1% SDS, 1% NP-40, 0.5% DOC, 50mM Tris, pH 7.5) (Wilson, I. et al., Id. 37:767 (1984)). Both cell lysate and culture media were precipitated with a HA specific monoclonal antibody. Proteins precipitated were analyzed on 15% SDS-PAGE gels.

Example 3

Cloning and expression of ETBR using the baculovirus expression system

The DNA sequence encoding the full length ETBR protein, ATCC # 75823, amplified using PCR oligonucleotide primers corresponding to the 5' and 3' sequences of the gene:

The 5' primer has the sequence 5'CGGGATCCGCCACCATGCGAGC CCCGGGCGCG 3' and contains a BamHI restriction enzyme site (in bold) followed by 6 nucleotides resembling an efficient signal for the initiation of translation in eukaryotic cells (J. Mol. Biol. 1987, 196, 947-950, Kozak, M.), and just behind, is the first 18 nucleotides of the ETBR gene (the initiation codon for translation "ATG" is underlined).

The 3' primer has the sequence 5'CGGGATCCCGCTCAGCAA TGAGTTCCGAC 3' and contains the cleavage site for the restriction endonuclease BamHI and 18 nucleotides complementary to the 3' non-translated sequence of the ETBR gene. The amplified sequences were isolated from a 1% agarose gel using a commercially available kit ("Geneclean," BIO 101 Inc., La Jolla, Ca.). The fragment was then digested with the endonucleases BamHI and then purified again on a 1% agarose gel. This fragment is designated F2.

The vector pRG1 (modification of pVL941 vector, discussed below) is used for the expression of the ETBR protein using the baculovirus expression system (for review see: Summers, M.D. and Smith, G.E. 1987, A manual of methods for baculovirus vectors and insect cell culture procedures, Texas Agricultural Experimental Station Bulletin No. 1555). This expression vector contains the strong polyhedrin promoter of the Autographa californica nuclear polyhedrosis virus (AcMNPV) followed by the recognition sites for the restriction endonucleases BamHI. The polyadenylation site of the simian virus (SV)40 is used for efficient polyadenylation. For an easy selection of recombinant viruses the beta-galactosidase gene from E.coli is inserted

in the same orientation as the polyhedrin promoter followed by the polyadenylation signal of the polyhedrin gene. The polyhedrin sequences are flanked at both sides by viral sequences for the cell-mediated homologous recombination of cotransfected wild-type viral DNA. Many other baculovirus vectors could be used in place of pRG1 such as pAc373, pVL941 and pAcIM1 (Luckow, V.A. and Summers, M.D., Virology, 170:31-39).

The plasmid was digested with the restriction enzymes BamHI and then dephosphorylated using calf intestinal phosphatase by procedures known in the art. The DNA was then isolated from a 1% agarose gel. This vector DNA is designated V2.

Fragment F2 and the dephosphorylated plasmid V2 were ligated with T4 DNA ligase. E.coli HB101 cells were then transformed and bacteria identified that contained the plasmid (pBacETBR) with the ETBR gene using the enzymes BamHI. The sequence of the cloned fragment was confirmed by DNA sequencing.

5 µg of the plasmid pBacETBR were cotransfected with 1.0 µg of a commercially available linearized baculovirus ("BaculoGold™ baculovirus DNA", Pharmingen, San Diego, CA.) using the lipofection method (Felgner et al. Proc. Natl. Acad. Sci. USA, 84:7413-7417 (1987)).

1µg of BaculoGold™ virus DNA and 5 µg of the plasmid pBacETBR were mixed in a sterile well of a microtiter plate containing 50 µl of serum free Grace's medium (Life Technologies Inc., Gaithersburg, MD). Afterwards 10 µl Lipofectin plus 90 µl Grace's medium were added, mixed and incubated for 15 minutes at room temperature. Then the transfection mixture was added dropwise to the Sf9 insect cells (ATCC CRL 1711) seeded in a 35 mm tissue culture plate with 1ml Grace' medium without serum. The plate was rocked back and forth to mix the newly added solution. The plate was then incubated for 5 hours at 27°C. After 5 hours the

transfection solution was removed from the plate and 1 ml of Grace's insect medium supplemented with 10% fetal calf serum was added. The plate was put back int an incubat r and cultivation continued at 27°C for four days.

After four days the supernatant was collected and a plaque assay performed similar as described by Summers and Smith (supra). As a modification an agarose gel with "Blue Gal" (Life Technologies Inc., Gaithersburg) was used which allows an easy isolation of blue stained plaques. (A detailed description of a "plaque assay" can also be found in the user's guide for insect cell culture and baculovirology distributed by Life Technologies Inc., Gaithersburg, page 9-10).

Four days after the serial dilution of the viruses was added to the cells, blue stained plaques were picked with the tip of an Eppendorf pipette. The agar containing the recombinant viruses was then resuspended in an Eppendorf tube containing 200 µl of Grace's medium. The agar was removed by a brief centrifugation and the supernatant containing the recombinant baculoviruses was used to infect Sf9 cells seeded in 35 mm dishes. Four days later the supernatants of these culture dishes were harvested and then stored at 4°C.

Sf9 cells were grown in Grace's medium supplemented with 10% heat-inactivated FBS. The cells were infected with the recombinant baculovirus V-ETBR at a multiplicity of infection (MOI) of 2. Six hours later the medium was removed and replaced with SF900 II medium minus methionine and cysteine (Life Technologies Inc., Gaithersburg). 42 hours later 5 µCi of ^{35}S-methionine and 5 µCi ^{35}S cysteine (Amersham) were added. The cells were further incubated for 16 hours before they were harvested by centrifugation and the labelled proteins visualized by SDS-PAGE and autoradiography.

Example 4

Xenopus Oocyte Assay to Identify Ligand

RNA was synthesized *in vitro* from lineariz d DNA, ATCC # 75823, using an RNA transcription kit. This RNA was microinjected into Xenopus oocytes (10ng of RNA/oocytes). The oocytes were manually defolliculated prior to microinjection to remove any endogenous receptors that might be present in the follicular membranes. The injected oocytes were maintained in modified Barth's medium at 18°C for 48 hours to allow for receptor protein expression. Electrophysiology was performed using the voltage-clamp technique. Oocytes were clamped at -60mV and the calcium activated chloride channel activity was recorded in Barth's medium at room temperature. Data were analyzed using Axotape software.

As shown in Figure 4, oocytes injected with the synthetic RNA complementary to DNA from ATCC # 75823, illicited fairly strong Cl^- currents upon addition of 10nM ET1, ET3 as well as Bombesin. Addition of ET1, ET3 and Bombesin to uninjected oocytes on the other hand did not elicit any change in membrane potential (data not shown). The ET1 and ET3 mediated response was blocked by the ET receptor peptide antagonist BQ123. Addition of related peptide ligands like AII, Neuropeptide Y and Bradykinin did not illicit any response (Figure 4). This indicates that the ETBR is functional and is capable of coupling to a second messenger system which leads to the mobilization of intercellular stores of calcium via production of inositol triphosphate. Since it responds to both ET and Bombesin it represents a novel endothelin-bombesin receptor.

Numerous modifications and variations of the present invention are possible in light of the above teachings and, therefore, within the scope of the appended claims, the invention may be practiced otherwise than as particularly described.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: LI, ET AL.

(ii) TITLE OF INVENTION: Human Endothelin Receptor

(iii) NUMBER OF SEQUENCES: 2

(iv) CORRESPONDENCE ADDRESS:

(A) ADDRESSEE: CARELLA, BYRNE, BAIN, GILFILLAN, CECCHI, STEWART & OLSTEIN

(B) STREET: 6 BECKER FARM ROAD

(C) CITY: ROSELAND

(D) STATE: NEW JERSEY

(E) COUNTRY: USA

(F) ZIP: 07068

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: 3.5 INCH DISKETTE

(B) COMPUTER: IBM PS/2

(C) OPERATING SYSTEM: MS-DOS

(D) SOFTWARE: WORD PERFECT 5.1

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER:

(B) FILING DATE: Concurrently

(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA

(A) APPLICATION NUMBER:

(B) FILING DATE:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: FERRARO, GREGORY D.
(B) REGISTRATION NUMBER: 36,134
(C) REFERENCE/DOCKET NUMBER: 325800-192

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 201-994-1700
(B) TELEFAX: 201-994-1744

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 4156 BASE PAIRS
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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CCCACTATGT TGGCCAGGAT GGTCTTGATT TCTTGACCTC GTGTTCTGCC CGCCTCTACC   60
TCCCAAAGTG CCGGGATTAC AGGCGTGACT GCTGTGCCCG GCCCCAGCAT CACTTTTATA  120
GCTTTCTGTG CCTCTTCCTC TGGGCCTTGG TGTATGAAGC CACTTGCCTT TCTCTGTTGG  180
GAAGCGAGCA GAATCAGATT GCTACTCATG ATGCAGTCCG GGCAGGGCAT ACTGTCACCT  240
TTGGCTGTGG ACACAGTTGT CAGGATAGGG GAGAAGCCCT TTAGGTCCGT CTTCTTGACA  300
CAGCCCTCCT ACCTGGTTAC GCTGGTGCTT TCGCTTGGTT TAGACAACCA AGACACTTGA  360
GAATTATGCT GTCCTCAGAA TGTCTGATGA AAAGAACAGA TTCACTTTTT GGACACAATG  420
CCCATTAGCC ATCTTTGGCA GTGTTTCTGA TCAAAGGTTC CCCATGCCTG CTCTAGGAAA  480
GTAAACTTTT TTCAGAATAA ATCCTCAAAT GGATTACTGA GTAGTCTTTG CACCATTCCC  540
ATCAGCCTAA TCAGACTGAA TGGTCACGCT CAGTGCAAAA AGCTGTTTTG CTGTTAGGAT  600
GTTTCAGTGT TTCTTGTCTT TCCTGGAACA GTTCAGTTGT TTAAATTTAG TAATTCAATC  660
CTGACCAGTG TAAACCCACT TAATTATTGC AGCCTAAAGA ATTCAGCTAC TTCTACTCTT  720
CATAAATGTG CCCAAGTAAA TATGTGTTTT TAATATTCAA CCCTGGAAAA TTAGTAATTC  780
AGATGATAAA AGCTCATGTT TTGGTGTCTT TGTACTCAGA TTGTGAACAG GCATATTTCA  840
CTGATTTAGA CTTAGTATAC TTGATGAGAA TGCTCAGGTT GAAGAGATAG TTCTGTCAGC  900
AATCCAACAT CTATAGCAAT GTGGAAAAAG TAATCAACTC ATATTTCACG AATTTGATGT  960
ATGTTGTGAT TTAGAGGGCA TGAGATAAAG TTTATATTTG AACTGTGTGG GGTAGGGGGA 1020
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AGAAGAGGTT GCTTAAGCAA ATGGGGGGGT GATTGAGGAA CAAGATGTCT CTAAGATGAG 1080
AAGTTATTTT CTTGCATCAT AGAAGCACTC TCTCCACCCG GGAGTGATTG TGTTAACTAT 1140
AAATCATTTA TATCTGTACA TTAAAGCAGA TTCCCTCAAT TAGGCAAATT TGGTTAGCCA 1200
AGCCCAAGTT ATTGTTTGTA CTTGAAAGTA ATAAAGCTGC ATTTCCTTAA AAATATATTC 1260
TGTAGTTAAG ACTTTGTCTT GCTTTCCGGA ATTCCTGTTT TTCTTTTCCT CTAGAGACCT 1320
CGGCTTGCAA CTGGATCAAA CGCTGTCGAA AGGATGTAAA TAGGCAGAGC AACTGTTACC 1380
AAGAAGGCCA CCACCCCCAC CCAAAGGCAG TGAGGAGTGT GGGGCTTCGT CTGGGCTCCC 1440
CCGAGTCTCA ACAGTAATCA ACAGTCAGGT GTTGATTGCA ACTTTTCAAG GTCAGCCACC 1500
GGGAGTAGCC TATTCCCTCT AGGAACCTTG GAGGGCATAC CTTGCTGGGA CTCAACTTGG 1560
CTGAGAAATG CACAAGATGC CAAAGGAGGA AGGATTATAG GGGGCGTGTG TGTGACCCCC 1620
AAGACCGATC TTCCGCTATC ACCCTAATCT CCGGTTCCCC GCTACCCGGG CGGGGGTGAG 1680
TATGTGACAT GTGCCTAACT CTCAGCAGCA ACTTCGGCAG CAGGTGTCGA TCCTAACTAA 1740
GCAGGAGCTG CGGCTGCCGG GTGTGCCCTC ACCAAGCCAT GCGAGCCCCG GGCGCGCTTC 1800
TCGCCCGCAT GTCGCGGCTA CTGCTTCTGC TACTGCTCAA GGTGTCTGCC TCTTCTGCCC 1860
TCGGGGTCGC CCCTGCGTCC AGAAACGAAA CTTGTCTGGG GGAGAGCTGT GCACCTACAG 1920
TGATCCAGCG CCGCGGCAGG GACGCCTGGG GACCGGGAAA TTCTGCAAGA GACGTTCTGC 1980
GAGCCCGAGC ACCCAGGGAG GAGCAGGGGG CAGCGTTTCT TGCGGGACCC TCCTGGGACC 2040
TGCCGGCGGC CCCGGACCGT GACCCGGCTG CAGGCAGAGG GGCGGAGGCG TCGACAGCCG 2100
GACCCCCGGG ACCTCCAACC AGGCCACCTG TCCCCTGGAG GTGGAAAGGT GCTCGGGGTC 2160
AGGAGCCTTC TGAAACTTTG GGGAGAGGGA ACCCCACGGC CCTCCAGCTC TTCCTTCAGA 2220
TCTCAGAGGA GGAAGAGAAG GGTCCCAGAG GCGCTGTCAT TTCCGGGCGT AGCCAGGAGC 2280
AGAGTGTGAA GACAGTCCCC GGAGCCAGCG ATCTTTTTTA CTGTCCAAGG AGAGCCGGGA 2340
AACTCCAGGG TTCCCACCAC AAGCCCCCAC CCAAGACGGC CAATGGACTG GCGGGGCACG 2400
AAGGGTGGAC AATTGCACTC CCGGGCCGGG CGCTGGCCCA GAATGGATCC TTGGGTGAAG 2460
GAATCCATGA TCCTGGGGGT CCCCGCCGGG GAAACAGCAC GAACCGGCGT GTGAGACTGA 2520
AGAACCCCTT CTACCCGCTG ACCCAGGAGT CCTATGGAGC CTACGCGGTC ATGTGTCTGT 2580
CCGTGGTGAT CTTCGGGACC GGCATCATTG GCAACCTGGC GGTGATGTGC ATCGTGTGCC 2640
ACAACTACTA CATGCGGAGC ATCTCCAACT CCCTCTTGGC CAACCTGGTC TTCTGGGACT 2700
TTCTCATCAT CTTCTTCTGC CTTCCGCTGG TCATCTTCCA CGAGCTGACC AAGAAGTGGC 2760
TGGTGGAGGA CTTCTCCTGC AAGATCGTGC CCTATATAGA GGTCGCTTCT CTGGGAGTCA 2820
CCACTTTCAC CTTATGTGCT CTGTGCATAG ACCGCTTCCG TGCTGCCACC AACGTACAGA 2880
TGTACTACGA AATGATCGAA AACTGTTCCT CAACAACTGC CAAACTTGCT GTTATATGGG 2940
TGGGAGCTCT ATTGTTAGCA CTTCCAGAAG TTGTTCTCCG CCAGCTGAGC AAGGAGGATT 3000
TGGGGTTTAG TGGCCGAGCT CCGGCAGAAA GGTGCATTAT TAAGATCTCT CCTGATTTAC 3060
CAGACACCAT CTATGTTCTA GCCCTCACCT ACGACAGTGC GAGACTGTGG TGGTATTTTG 3120
GCTGTTACTT TTGTTTGCCC ACGCTTTTCA CCATCACCTG CTCTCTAGTG ACTGCGAGGA 3180
AAATCCGCAA AGCAGAGAAA GCCTGTACCC GAGGGAATAA ACGGCAGATT CAACTAGAGA 3240
GTCAGATGAA CTGTACAGTA GTGGCACTGA CCATTTTATA TGGATTGGGC ATTATTCCTG 3300
AAAATATCTG CAACATTGTT ACTGCCTACA TGGCTACAGG GGTTTCACAG CAGACAATGG 3360
ACCTCCTTAA TATCATCAGC CAGTTCCTTT TGTTCTTTAA GTCCTGTGTC ACCCCAGTCC 3420
TCCTTTTCTG TCTCTGCAAA CCCTTCAGTC GGGCCTTCAT GGAGTGCTGC TGCTGTTGCT 3480
GTGAGGAATG CATTCAGAAG TCTTCAACGG TGACCAGTGA TGACAATGAC AACGAGTACA 3540
CCACGGAACT CGAACTCTCG CCTTTCAGTG CCATACGCCG TGAAATGTCC ACTTTTGCTT 3600

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CTGTCGGAAC TCATTGCTGA AGGACAGTAC TTGGTTGGGT CAGATTTATT TGTTTGATTT  3660
TCATATCCCG TGAAAGTTTT TAATTCATAT TTTTCCTTAT AGGGAAAAAT GCAAAAAAGA  3720
AACAATAAAG AAAGAAATAT TAACTACTGT AGAACTGATT TTACAAATTA ATATTTGTGC  3780
TTTGAAAAAA AGTTTCTATT TAGTTATTTA AGAAGAATGA GAAGGCCAAT AGTTTTAGAT  3840
TATTTTATCT GGTATGGTGC TAATATTTTA TTTGAAAAAA GTTACTGCAA CTTAACTTAA  3900
AATTGCTAAC GTTTTTTCTT CTTTTAAAAA TACAATTATT GTATATTAAT TATAGCAATG  3960
TGATTTTGTA GGTTATTTTA TATTTGAGTT GTGATTGAAA GTATGTTGTA TATGGTATTG  4020
TGAGATGATT TGTACTTGGA AGCATTCACA AAGTAGCACC AAATAAATTA CACTTTATTC  4080
TTTAATGTCA TTGTCAATCT ACTTTTAACC AATATTCAAT AAATCTTCTA ATTGCCTTAA  4140
AAAAAAAAAA AAAAAA                                                  4156
```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 613 AMINO ACIDS

(B) TYPE: AMINO ACID

(C) STRANDEDNESS:

(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: PROTEIN

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```
Met Arg Ala Pro Gly Ala Leu Leu Ala Arg Met Ser Arg Leu Leu
  -25                 -20                 -15
Leu Leu Leu Leu Leu Lys Val Ser Ala Ser Ser Ala Leu Gly Val
  -10                 -5                  1
Ala Pro Ala Ser Arg Asn Glu Thr Cys Leu Gly Glu Ser Cys Ala
5                     10                  15
Pro Thr Val Ile Gln Arg Arg Gly Arg Asp Ala Trp Gly Pro Gly
20                    25                  30
Asn Ser Ala Arg Asp Val Leu Arg Ala Arg Ala Pro Arg Glu Glu
35                    40                  45
Gln Gly Ala Ala Phe Leu Ala Gly Pro Ser Trp Asp Leu Pro Ala
50                    55                  60
Ala Pro Asp Arg Asp Pro Ala Ala Gly Arg Gly Ala Glu Ala Ser
65                    70                  75
```

Thr Ala Gly Pro Pro Gly Pro Pro Thr Arg Pr Pro Val Pro Trp
80 85 90
Arg Trp Lys Gly Ala Arg Gly Gln Glu Pro Ser Glu Thr Leu Gly
95 100 105
Arg Gly Asn Pro Thr Ala Leu Gln Leu Phe Leu Gln Ile Ser Glu
110 115 120
Glu Glu Glu Lys Gly Pro Arg Gly Ala Val Ile Ser Gly Arg Ser
125 130 135
Gln Glu Gln Ser Val Lys Thr Val Pro Gly Ala Ser Asp Leu Phe
140 145 150
Tyr Cys Pro Arg Arg Ala Gly Lys Leu Gln Gly Ser His His Lys
155 160 165
Pro Leu Ser Lys Thr Ala Asn Gly Leu Ala Gly His Glu Gly Trp
170 175 180
Thr Ile Ala Leu Pro Gly Arg Ala Leu Ala Gln Asn Gly Ser Leu
185 190 195
Gly Glu Gly Ile His Asp Pro Gly Gly Pro Arg Arg Gly Asn Ser
200 205 210
Thr Asn Arg Arg Val Arg Leu Lys Asn Pro Phe Tyr Pro Leu Thr
215 220 225
Gln Glu Ser Tyr Gly Ala Tyr Ala Val Met Cys Leu Ser Val Val
230 235 240
Ile Phe Gly Thr Gly Ile Ile Gly Asn Leu Ala Val Met Cys Ile
245 250 255
Val Cys His Asn Tyr Tyr Met Arg Ser Ile Ser Asn Ser Leu Leu
260 265 270
Ala Asn Leu Val Phe Trp Asn Phe Leu Ile Ile Phe Phe Cys Leu
275 280 285
Pro Leu Val Ile Phe His Gly Leu Thr Lys Lys Trp Leu Val Glu
290 295 300
Asp Phe Ser Cys Lys Ile Val Pro Tyr Ile Glu Val Ala Ser Leu
305 310 315
Gly Val Thr Thr Phe Thr Leu Cys Ala Leu Cys Ile Asp Arg Phe
320 325 330

Arg Ala Ala Thr Asn Val Gln Met Tyr Tyr Glu Met Il Glu Asn
335 340 345
Cys Ser Ser Thr Thr Ala Lys Leu Ala Val Ile Trp Val Gly Ala
350 355 360
Leu Leu Leu Ala Leu Pro Glu Val Val Leu Arg Gln Leu Ser Lys
365 370 375
Glu Asp Leu Gly Phe Ser Gly Arg Ala Pro Ala Glu Arg Cys Ile
380 385 390
Ile Lys Ile Ser Pro Asp Leu Pro Asp Thr Ile Tyr Val Leu Ala
395 400 405
Leu Thr Tyr Asp Ser Ala Arg Lys Trp Trp Tyr Phe Gly Cys Tyr
410 415 420
Phe Cys Leu Pro Thr Leu Phe Thr Ile Thr Cys Ser Leu Val Thr
425 430 435
Ala Arg Lys Ile Arg Lys Ala Glu Lys Ala Cys Thr Arg Gly Asn
440 445 450
Lys Arg Gln Ile Gln Leu Glu Ser Gln Met Asn Cys Thr Val Val
455 460 465
Ala Leu Thr Ile Leu Tyr Gly Leu Gly Ile Ile Pro Glu Asn Ile
470 475 480
Cys Asn Ile Val Thr Ala Tyr Met Ala Thr Gly Val Ser Gln Gln
485 490 495
Thr Met Asp Leu Leu Asn Ile Ile Ser Gln Phe Leu Leu Phe Phe
500 505 510
Lys Ser Cys Val Thr Pro Val Leu Leu Phe Cys Leu Cys Lys Pro
515 520 525
Phe Ser Arg Ala Phe Met Glu Cys Cys Cys Cys Cys Cys Glu Glu
530 535 540
Cys Ile Gln Lys Ser Ser Thr Val Thr Ser Asp Asp Asn Asp Asn
545 550 555
Glu Tyr Thr Thr Glu Leu Glu Leu Ser Pro Phe Ser Ala Ile Arg
560 565 570
rg Glu Met Ser Thr Phe Ala Ser Val Gly Thr His Cys
575 580 585

WHAT IS CLAIMED IS:

1. An isolated polynucleotide selected from the group consisting of:

(a) a polynucleotide encoding the G-protein coupled receptor polypeptide having the deduced amino acid sequence of Figure 1 or a fragment, analog or derivative of said polypeptide;

(b) a polynucleotide encoding the G-protein coupled receptor polypeptide having the amino acid sequence encoded by the cDNA contained in ATCC Deposit No. 75823 or a fragment, analog or derivative of said polypeptide.

2. The polynucleotide of Claim 1 wherein the polynucleotide is DNA.

3. The polynucleotide of Claim 1 wherein the polynucleotide is RNA.

4. The polynucleotide of Claim 1 wherein the polynucleotide is genomic DNA.

5. The polynucleotide of Claim 2 wherein said polynucleotide encodes G-protein coupled receptor having the deduced amino acid sequence of Figure 1.

6. The polynucleotide of Claim 2 wherein said polynucleotide encodes the G-protein coupled receptor polypeptide encoded by the cDNA of ATCC Deposit No. 75823.

7. The polynucleotide of Claim 1 having the coding sequence of G-protein coupled receptor as shown in Figure 1.

8. The polynucleotide of Claim 2 having the coding sequence of G-protein coupled receptor deposited as ATCC Deposit No. 75823.

9. A vector containing the DNA of Claim 2.

10. A host cell genetically engineered with the vector of Claim 9.

11. A process for producing a polypeptide comprising: expressing from the host cell of Claim 10 the polypeptide encoded by said DNA.

12. A process for producing cells capable f expressing a polypeptide comprising genetically engineering cells with the vector of Claim 9.

13. An isolated DNA hybridizable to the DNA of Claim 2 and encoding a polypeptide having G-protein coupled receptor activity.

14. A polypeptide selected from the group consisting of (i) a G-protein coupled receptor polypeptide having the deduced amino acid sequence of Figure 1 and fragments, analogs and derivatives thereof and (ii) a G-protein coupled receptor polypeptide encoded by the cDNA of ATCC Deposit No. 75823 and fragments, analogs and derivatives of said polypeptide.

15. The polypeptide of Claim 14 wherein the polypeptide is a G-protein coupled receptor having the deduced amino acid sequence of Figure 1.

16. An antibody against the polypeptide of claim 14.

17. A compound which activates the G-protein coupled receptor polypeptide of claim 14.

18. A compound which inhibits activation of the G-protein coupled receptor polypeptide of claim 14.

19. A compound which inhibits bombesin activation of the G-protein coupled receptor polypeptide of claim 14.

20. A method for the treatment of a patient having need of activation of a G-protein coupled receptor polypeptide of claim 14 comprising: administering to the patient a therapeutically effective amount of the compound of Claim 17.

21. A method for the treatment of a patient having need to inhibit activation of-a G-protein coupled receptor of claim 14 comprising: administering to the patient a therapeutically effective amount of the compound of Claim 18.

22. A method for the treatment of a patient having need to inhibit activation of a G-protein coupled receptor

of claim 14 comprising: administering t the patient a therapeutically eff ctive amount f a compound which inhibits activation of the G-protein coupled rec ptor by bombesin.

23. The polypeptide of Claim 14 wherein the polypeptide is a soluble fragment of the G-protein coupled receptor and is capable of binding a ligand for the receptor.

24. A process for identifying antagonists and agonists to the G-protein coupled receptor comprising:

providing appropriate cells which express the receptor on the surface thereof;

contacting the cell with a receptor ligand and a compound to be screened;

determining the signal generated by the cell in response to binding of the ligand; and

identifying antagonists or agonists to the G-protein coupled receptor.

25. A process for determining whether a ligand not known to be capable of binding to a G-protein coupled receptor can bind thereto comprising:

contacting a mammalian cell which expresses the G-protein coupled receptor with a potential ligand;

detecting the presence of the ligand which binds to the receptor; and

determining whether the ligand binds to the G-protein coupled receptor.

FIG. 1A

```
10                  30                  50
CCCACTATGTTGGCCAGGATGGTCTTGATTTCTTGACCTCGTGTTCTGCCCGCCTCTACC
   70                  90                 110
TCCCAAAGTGCCGGGATTACAGGCGTGACTGCTGTGCCCGGCCCCAGCATCACTTTTATA
  130                 150                 170
GCTTTCTGTGCCTCTCTTCCTCTGGGCCTTGGTGTATGAAGCCACTTGCCTTTCTCTGTTGG
  190                 210                 230
GAAGCGAGCAGAATCAGATTGCTACTCATGATGCAGTCCGGGCAGGGCATACTGTCACCT
  250                 270                 290
TTGGCTGTGTGGACACAGTTGTCAGGATAGGGGAGAAGCCCTTTAGGTCCGTCTTCTTGACA
  310                 330                 350
CAGCCCTCCTACCTGGTTACGCTGGTGCTTTCGCTTGGTTTAGACAACCAAGACACTTGA
  370                 390                 410
GAATTATGCTGTCCTCAGAATGTCTGATGAAAAGAACAGATTCACTTTTTGGACACAATG
  430                 450                 470
CCCATTAGCCATCTTTGGCAGTGTTTCTGATCAAAGGTTCCCCATGCCTGCTCTAGGAAA
  490                 510                 530
GTAAACTTTTTTCAGAATAAATCCTCAAATGGATTACTGAGTAGTCTTTGCACCATTCCC
  550                 570                 590
ATCAGCCTAATCAGACTGAATGGTCACGCTCAGTGCAAAAAGCTGTTTTGCTGTTTAGGAT
  610                 630                 650
```

MATCH WITH FIG. 1B

FIG. 1B

MATCH WITH FIG. 1A

GTTTCAGTGTTTCTTGTCTTTCCTGGAACAGTTCAGTTGTTTAAATTTAGTAATTCAATC
670 690 710

CTGACCAGTGTAAACCCACTTAATTATTGCAGCCTAAAGAATTCAGCTACTTCTACTCTT
730 750 770

CATAAATGTGCCCAAGTAAATATGTGTTTTTAATATTCAACCCTGGAAAATTAGTAATTC
790 810 830

AGATGATAAAAGCTCATGTTTTGGTGTCTTTGTACTCAGATTGTGAACAGGCATATTTCA
850 870 890

CTGATTTAGACTTAGTATACTTGATGAGAATGCTCAGGTTGAAGAGATAGTTCTGTCAGC
910 930 950

AATCCAACATCTATAGCAATGTGGAAAAAGTAATCAACTCATATTTCACGAATTTGATGT
970 990 1010

ATGTTGTGATTTAGAGGGCATGAGATAAAGTTTATATTTGAACTGTGTGGGGTAGGGGGA
1030 1050 1070

AGAAGAGGTTGCTTAAGCAAATGGGGGGGTGATTGAGGAACAAGATGTCTCTAAGATGAG
1090 1110 1130

AAGTTATTTTCTTGCATCATAGAAGCACTCTCTCCACCCGGGAGTGATTGTGTTAACTAT
1150 1170 1190

AAATCATTTATATCTGTACATTAAAGCAGATTCCCTCAATTAGGCAAATTTGGTTAGCCA
1210 1230 1250

MATCH WITH FIG. 1C

FIG. 1C

MATCH WITH FIG. 1B

AGCCCAAGTTATTGTTGTACTTGAAAGTAATAAAGCTGCATTTCCTTAAAAATATATTC
1270 1290 1310

TGTAGTTAAGACTTTGTCTTGCTTTCCGGAATTCCTGTTTTTCTTTTCCTCTAGAGACCT
1330 1350 1370

CGGCTTGCAACTGGATCAAACGCTGTCGAAAGGATGTAAATAGGCAGAGCAACTGTTACC
1390 1410 1430

AAGAAGGCCACCACCCCCACCCCAAAGGCAGTGAGGAGTGTGGGGCTTCGTCTGGGCTCCC
1450 1470 1490

CCGAGTCTCAACAGTAATCAACAGTCAGGTGTTGATTGCAACTTTTCAAGGTCAGCCACC
1510 1530 1550

GGGAGTAGCCTATTCCCTCTAGGAACCTTGGAGGGCATACCTTGCTGGGACTCAACTTGG
1570 1590 1610

CTGAGAAATGCACACAAGATGCCAAAGGAGGAAGGATTATAGGGGGCGTGTGTGTGACCCCC
1630 1650 1670

AAGACCGATCTTCCGCTATCACCCTAATCTCCGGTTCCCCGCTACCCGGGCGGGGGTGAG
1690 1710 1730

TATGTGACATGTGCCTAACTCTCAGCAGCAACTTCGGCAGGCAGGTGTCGATCCTAACTAA
1750 1770 1790

GCAGGAGCTGCGGCTGCCGGGTGTGCCCTCACCAAGCCATGCGAGCCCCGGGCGCGCTTC

MATCH WITH FIG. 1D

MATCH WITH FIG. IC

FIG. ID

```
                                                  M  R  A  P  G  A  L  L
      1810                  1830                        1850
      .                     .                           .
TCGCCCGCATGTCGCGGCTACTGCTTCTGCTACTGCTCAAGGTGTCTGCCTCTTCTGCCC
  A  R  M  S  R  L  L  L  L  L  L  L  K  V  S  A  S  S  A  L
      1870                  1890                        1910
      .                     .                           .
TCGGGGTCGCCCCTGCGTCCAGAAACGAAACTTGTCTGGGGGAGAGCTGTGCACCTACAG
  G  V  A  P  A  S  R  N  E  T  C  L  G  E  S  C  A  P  T  V
      1930                  1950                        1970
      .                     .                           .
TGATCCAGCGCCGCGGCAGGGACGCCTGGGGACCGGGAAATTCTGCAAGAGACGTTCTGC
  I  Q  R  R  G  R  D  A  W  G  P  G  N  S  A  R  D  V  L  R
      1990                  2010                        2030
      .                     .                           .
GAGCCCGAGCACCCAGGGAGGAGCAGGGGGCAGCGTTTCTTGCGGGACCCTCCTGGGACC
  A  R  A  P  R  E  E  Q  G  A  A  F  L  A  G  P  S  W  D  L
      2050                  2070                        2090
      .                     .                           .
TGCCGGCGGCCCCGGACCGTGACCCGGCTGCAGGCAGAGGGGCGGAGGCGTCGACAGCCG
  P  A  A  P  D  R  D  P  A  A  G  R  G  A  E  A  S  T  A  G
      2110                  2130                        2150
      .                     .                           .
GACCCCCGGGACCTCCAACCAGGCCACCTGTCCCCTGGAGGTGGAAAGGTGCTCGGGGTC
  P  P  G  P  P  T  R  P  P  V  P  W  R  W  K  G  A  R  G  Q
      2170                  2190                        2210
      .                     .                           .
AGGAGCCTTCTGAAACTTTGGGGAGAGGGAACCCCACGGCCCTCCAGCTCTTCCTTCAGA
  E  P  S  E  T  L  G  R  G  N  P  T  A  L  Q  L  F  L  Q  I
      2230                  2250                        2270
```

MATCH WITH FIG. IE

FIG. 1E

MATCH WITH FIG. 1D

```
TCTCAGAGGAGGAAGAGAAGGGTCCCAGAGGCGCTGTCATTTCCGGGCGTAGCCAGGAGC
  S  E  E  E  K  G  P  R  G  A  V  I  S  G  R  S  Q  E  Q
     2290          2310          2330

AGAGTGTGAAGACAGTCCCCGGAGCCAGCGATCTTTTTTACTGTCCAAGGAGAGCCGGGA
  S  V  K  T  V  P  G  A  S  D  L  F  Y  C  P  R  R  A  G  K
     2350          2370          2390

AACTCCAGGGTTCCCACCACAAGCCCCTGTCCAAGACGGCCAATGGACTGGCGGGGCACG
  L  Q  G  S  H  H  K  P  L  S  K  T  A  N  G  L  A  G  H  E
     2410          2430          2450

AAGGGTGGACAATTGCACTCCCGGGCCGGCGCTGGCCCAGAATGGATCCTTGGGTGAAG
  G  W  T  I  A  L  P  G  R  A  L  A  Q  N  G  S  L  G  E  G
     2470          2490          2510

GAATCCATGATCCTGGGGGTCCCCGCCGGGAAACAGCACGAACCGGCGTGTGAGACTGA
  I  H  D  P  G  G  P  R  R  G  N  S  T  N  R  R  V  R  L  K
     2530          2550          2570

AGAACCCCTTCTACCCGCTGACCCAGGAGTCCTATGGAGCCTACGCGGTCATGTGTCTGT
  N  P  F  Y  P  L  T  Q  E  S  Y  G  A  Y  A  V  M  C  L  S
     2590          2610          2630

CCGTGGTGATCTTCGGGACCGGCATCATTGGCAACCTGGCGGTGATGTGCATCGTGTGCC
  V  V  I  F  G  T  G  I  I  G  N  L  A  V  M  C  I  V  C  H
     2650          2670          2690

ACAACTACTACATGCGGAGCATCTCCAACTCCCTCTTGGCCAACCTGGTCTTCTGGGACT
```

MATCH WITH FIG. 1F

FIG. 1F

```
MATCH WITH FIG. 1E
N Y Y M R S I S N S L L A N L V F W D F
  2710      2730      2750
TTCTCATCATCTTCTTCTGCCTTCCGCTGGTCATCTTCCACGAGCTGACCAAGAAGTGGC
 L I I F F C L P L V I F H E L T K K W L
    2770      2790      2810
TGGTGGAGGACTTCTCCTGCAAGATCGTGCCCTATATAGAGGTCGCTTCTCTGGGAGTCA
 V E D F S C K I V P Y I E V A S L G V T
    2830      2850      2870
CCACTTTCACCTTATGTGCTCTGTGCATAGACCGCTTCCGTGCTGCCACCAACGTACAGA
 T F T L C A L C I D R F R A A T N V Q M
    2890      2910      2930
TGTACTACGAAATGATCGAAAACTGTTCCTCAACACTGCCAAACTTGCTGTTATATGGG
 Y Y E M I E N C S S T T A K L A V I W V
    2950      2970      2990
TGGGAGCTCTATTGTTAGCACTTCCAGAAGTTGTTCTCCGCCAGCTGAGCAAGGAGGATT
 G A L L L A L P E V V L R Q L S K E D L
    3010      3030      3050
TGGGGTTTAGTGGCCGAGCTCCGGCAGAAAGGTGCATTATTAAGATCTCTCCTGATTTAC
 G F S G R A P A E R C I I K I S P D L P
    3070      3090      3110
CAGACACCATCTATGTTCTAGCCCTCACCTACGACAGTGCGAGACTGTGGTGGTATTTTG
 D T I Y V L A L T Y D S A R L W W Y F G
    3130      3150      3170
MATCH WITH FIG. 1G
```

FIG. 1G

```
MATCH WITH FIG. 1F
GCTGTTACTTTTGTTTGCCCACGCTTTTCACCATCACCTGCTCTCTAGTGACTGCGGAGGA
  C  Y  F  C  L  P  T  L  F  T  I  T  C  S  L  V  T  A  R  K
     3190          3210          3230
AAATCCGCAAAGCAGAGAAAGCCTGTACCCGAGGGAATAAACGGCAGATTCAACTAGAGA
  I  R  K  A  E  K  A  C  T  R  G  N  K  R  Q  I  Q  L  E  S
     3250          3270          3290
GTCAGATGAACTGTACAGTAGTGGCACTGACCATTTTATATGGATTGGGCATTATTCCTG
  Q  M  N  C  T  V  V  A  L  T  I  L  Y  G  L  G  I  I  P  E
     3310          3330          3350
AAAATATCTGCAACATTGTTACTGCCTACATGGCTACAGGGGTTTCACAGCAGACAATGG
  N  I  C  N  I  V  T  A  Y  M  A  T  G  V  S  Q  Q  T  M  D
     3370          3390          3410
ACCTCCTTAATATCATCAGCCAGTTCCTTTTGTTCTTTAAGTCCTGTGTCACCCCAGTCC
  L  L  N  I  I  S  Q  F  L  L  F  F  K  S  C  V  T  P  V  L
     3430          3450          3470
TCCTTTTCTGTCTCTGCAAACCCTTCAGTCGGGCCTTCATGGAGTGCTGCTGTTGCT
  L  F  C  L  C  K  P  F  S  R  A  F  M  E  C  C  C  C  C
     3490          3510          3530
GTGAGGAATGCATTCAGAAGTCTTCAACGGTGACCAGTGATGACAATGACGAGTACA
  E  E  C  I  Q  K  S  S  T  V  T  S  D  D  N  D  N  E  Y  T
     3550          3570          3590
CCACGGAACTCGAACTCTCGCCTTTCAGTGCCATACGCCGTGAAATGTCCACTTTTGCTT
MATCH WITH FIG. 1H
```

FIG. 1H

MATCH WITH FIG. 1G

```
T  E  L  E  L  S  P  F  S  A  I  R  R  E  M  S  T  F  A  S
3610                3630                3650

CTGTCGGAACTCATTGCTGAAGGACAGTACTTGGTTGGGTCAGATTTATTTGTTTGATTT
V  G  T  H  C  *
3670                3690                3710

TCATATCCCGTGAAAGTTTTTAATTCATATTTTTCCTTATAGGGAAAAATGCAAAAAAGA
3730                3750                3770

AACAATAAAGAAAGAAATATTAACTACTGTAGAACTGATTTTACAAATTAATATTTGTGC
3790                3810                3830

TTTGAAAAAAAGTTTCTATTTAGTTATTTAAGAAGAATGAGAAGGCCAATAGTTTTAGAT
3850                3870                3890

TATTTTATCTGGTATGGTGCTAATATTTTATTTGAAAAAAGTTACTGCAACTTAACTTAA
3910                3930                3950

AATTGCTAACGTTTTTTCTTCTTTTAAAAATACAATTATTGTATATTAATTATAGCAATG
3970                3990                4010

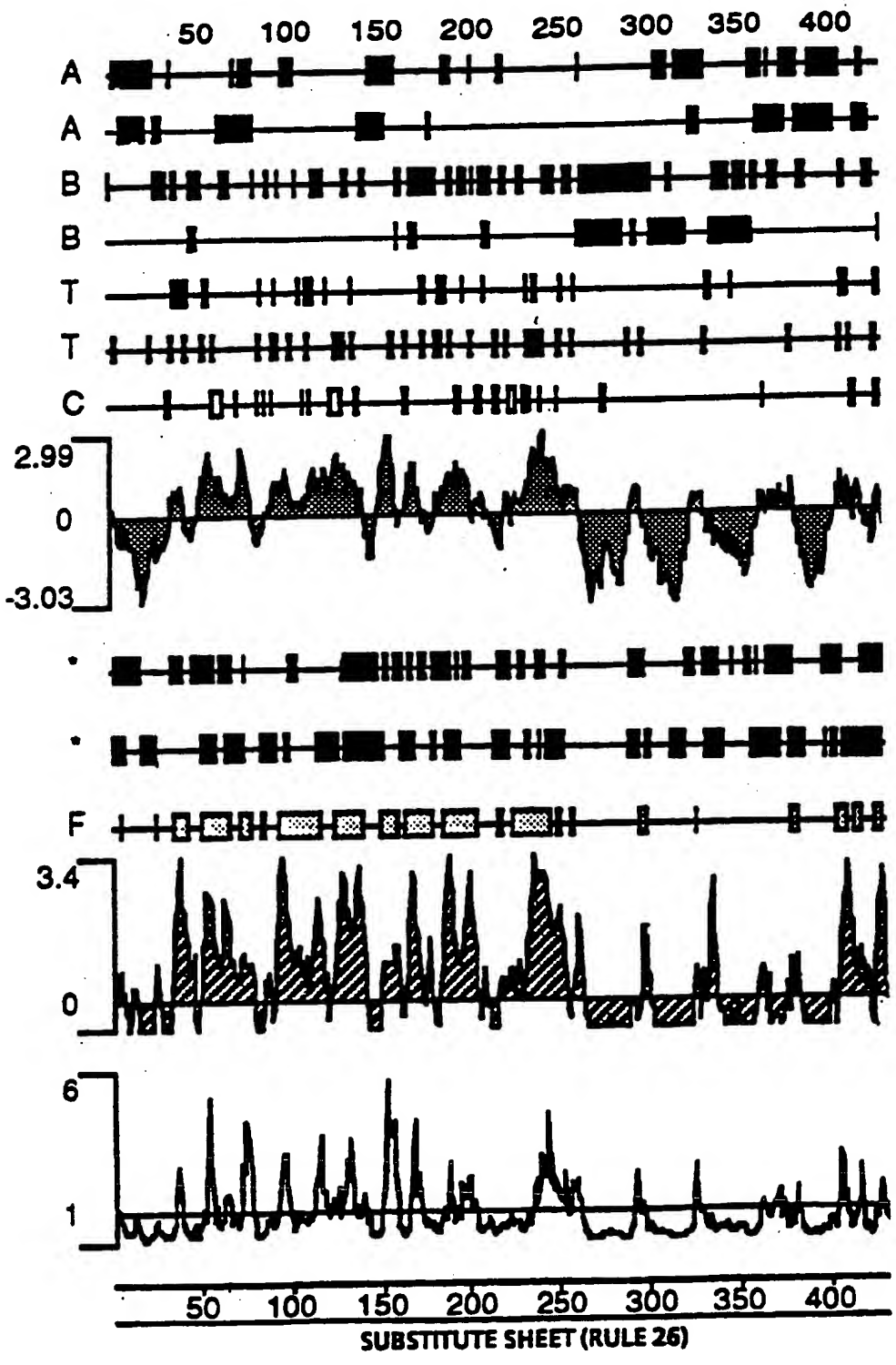
TGATTTTGTAGGTTATTTTATATTTGAGTTGTGATTGAAAGTATGTTGTATATGGTATTG
4030                4050                4070

TGAGATGATTTGTACTTGGAAGCATTCACAAAGTAGCACCAAATAAATTACACTTTATTC
4090                4110                4130

TTTAATGTCATTGTCAATCTACTTTTAACCAATATTCAATAAATCTTCTAATTGCCTTAA
4150

AAAAAAAAAAAAAA
```

FIG. 2A



MATCH WITH FIG. 2B

FIG. 2B

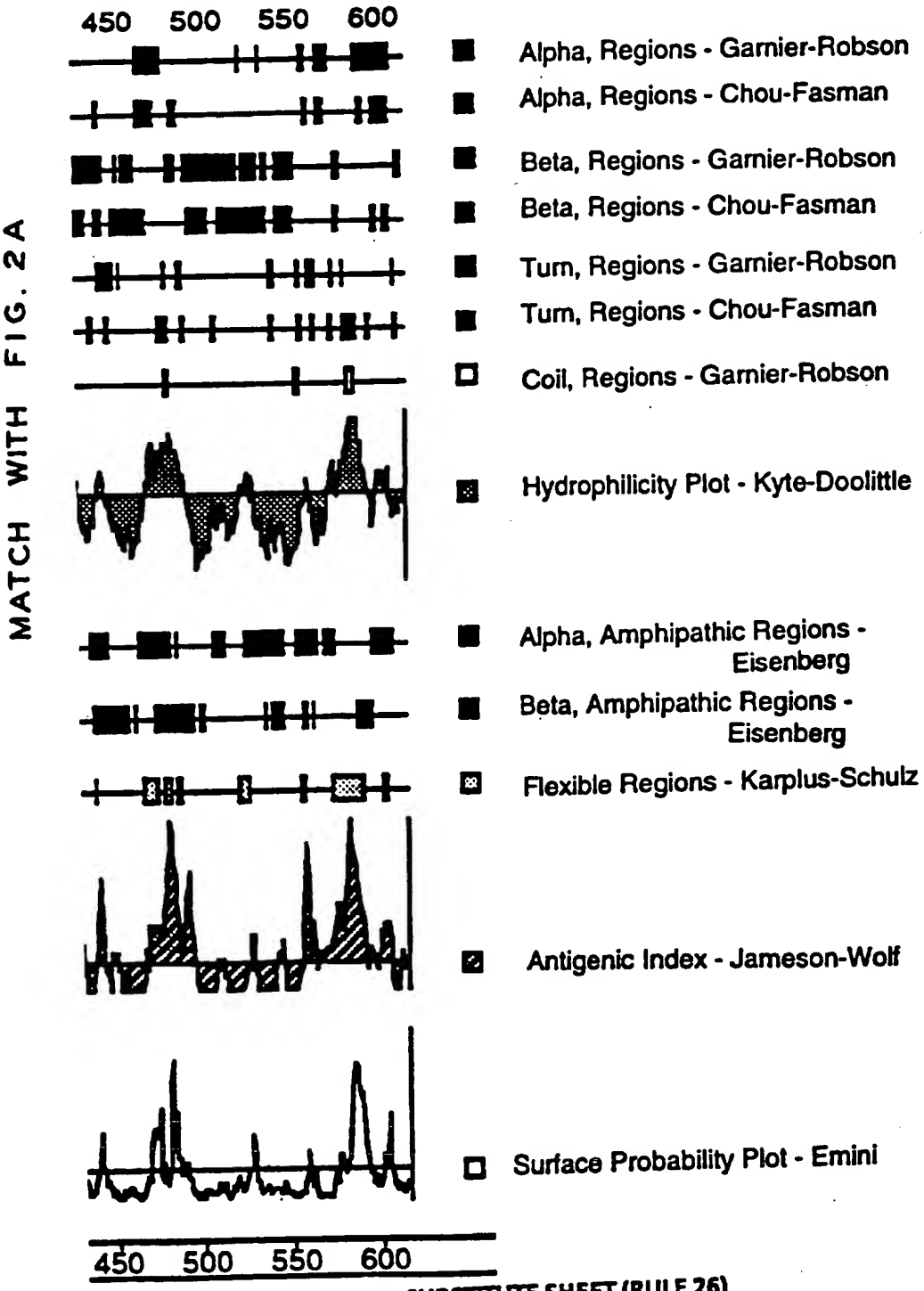
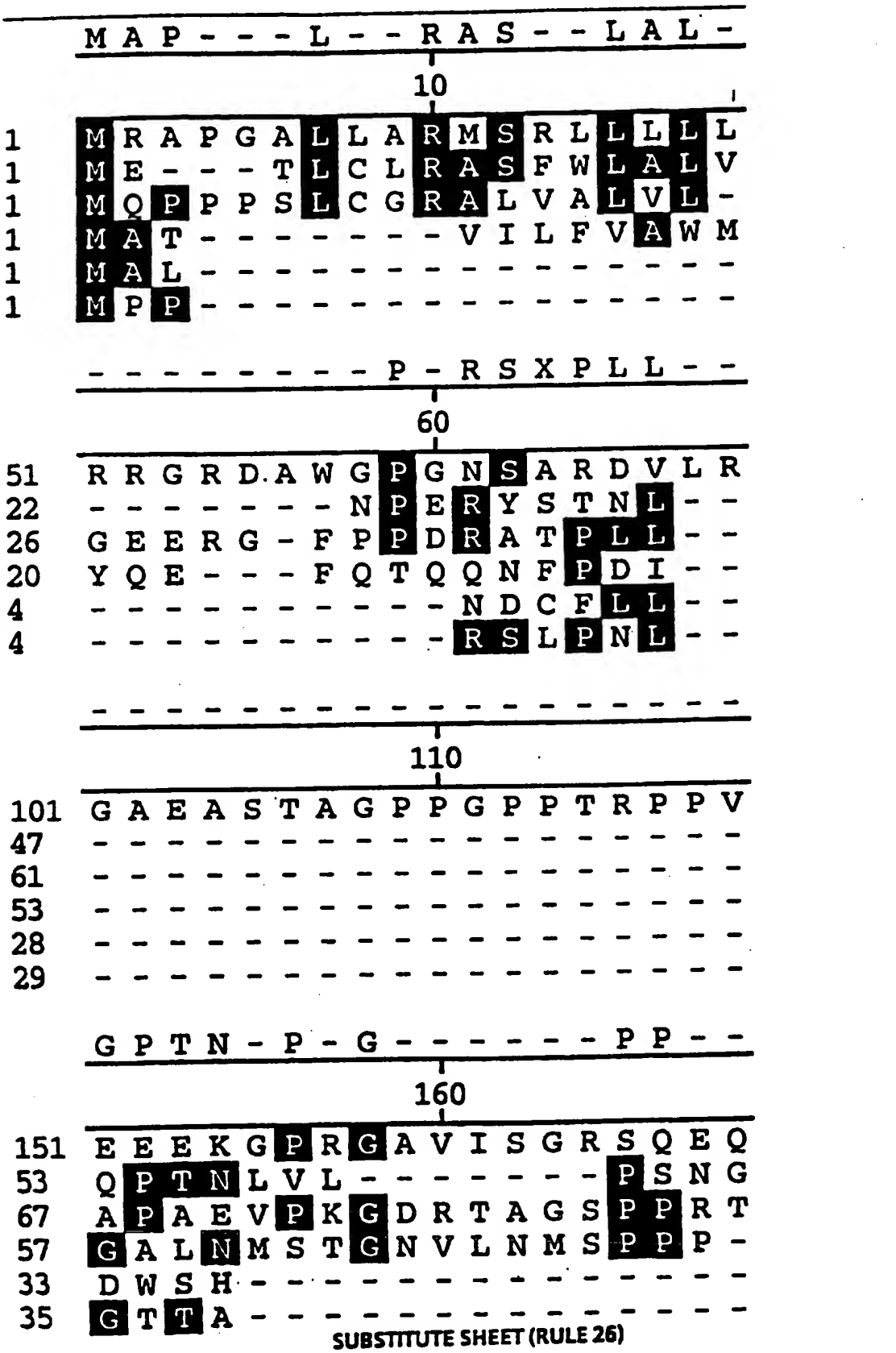
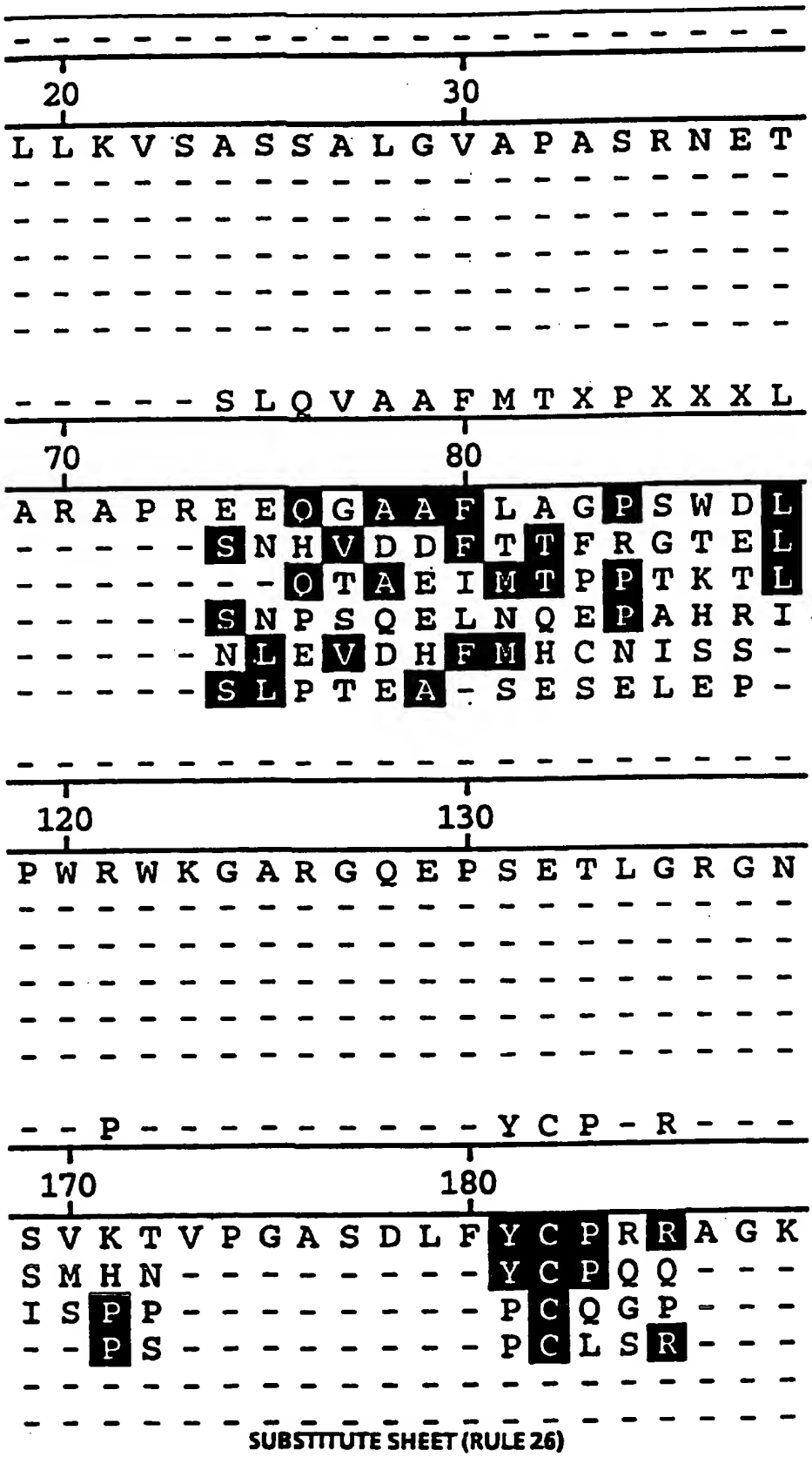

450
500
550
600
MATCH WITH FIG. 2A
Alpha, Regions - Garnier-Robson
Alpha, Regions - Chou-Fasman
Beta, Regions - Garnier-Robson
Beta, Regions - Chou-Fasman
Turn, Regions - Garnier-Robson
Turn, Regions - Chou-Fasman
Coil, Regions - Garnier-Robson
Hydrophilicity Plot - Kyte-Doolittle
Alpha, Amphipathic Regions - Eisenberg
Beta, Amphipathic Regions - Eisenberg
Flexible Regions - Karplus-Schulz
Antigenic Index - Jameson-Wolf
Surface Probability Plot - Emini
450
500
550
600

FIG. 3Aα 11/21 MATCH WITH FIG. 3Ab



MATCH WITH FIG. 3Aa 12/21 MATCH WITH FIG. 3Ac

FIG. 3Ab



MATCH WITH FIG. 3Ab

FIG. 3Ac

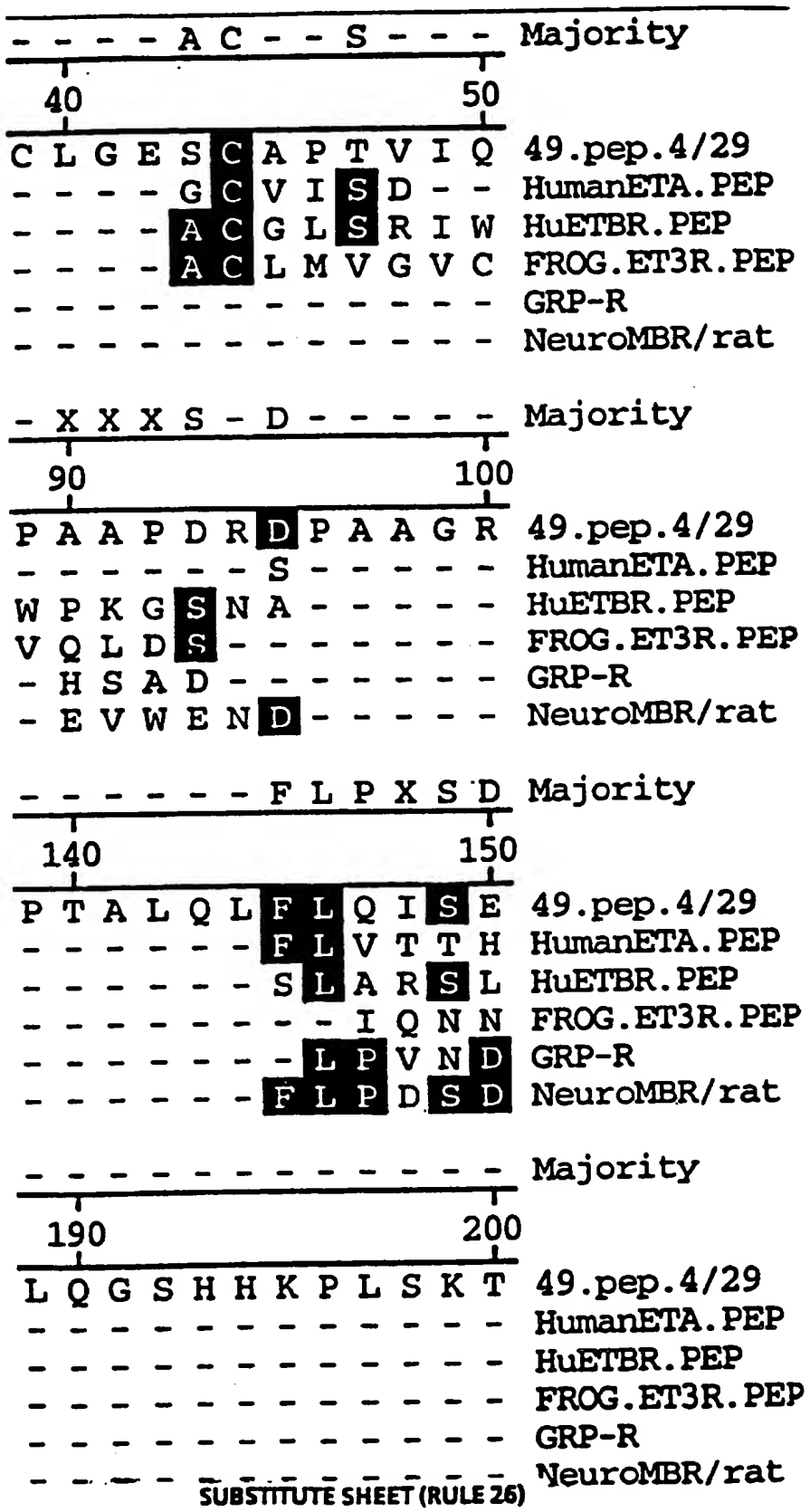


FIG. 3Ba

MATCH WITH FIG. 3Bb

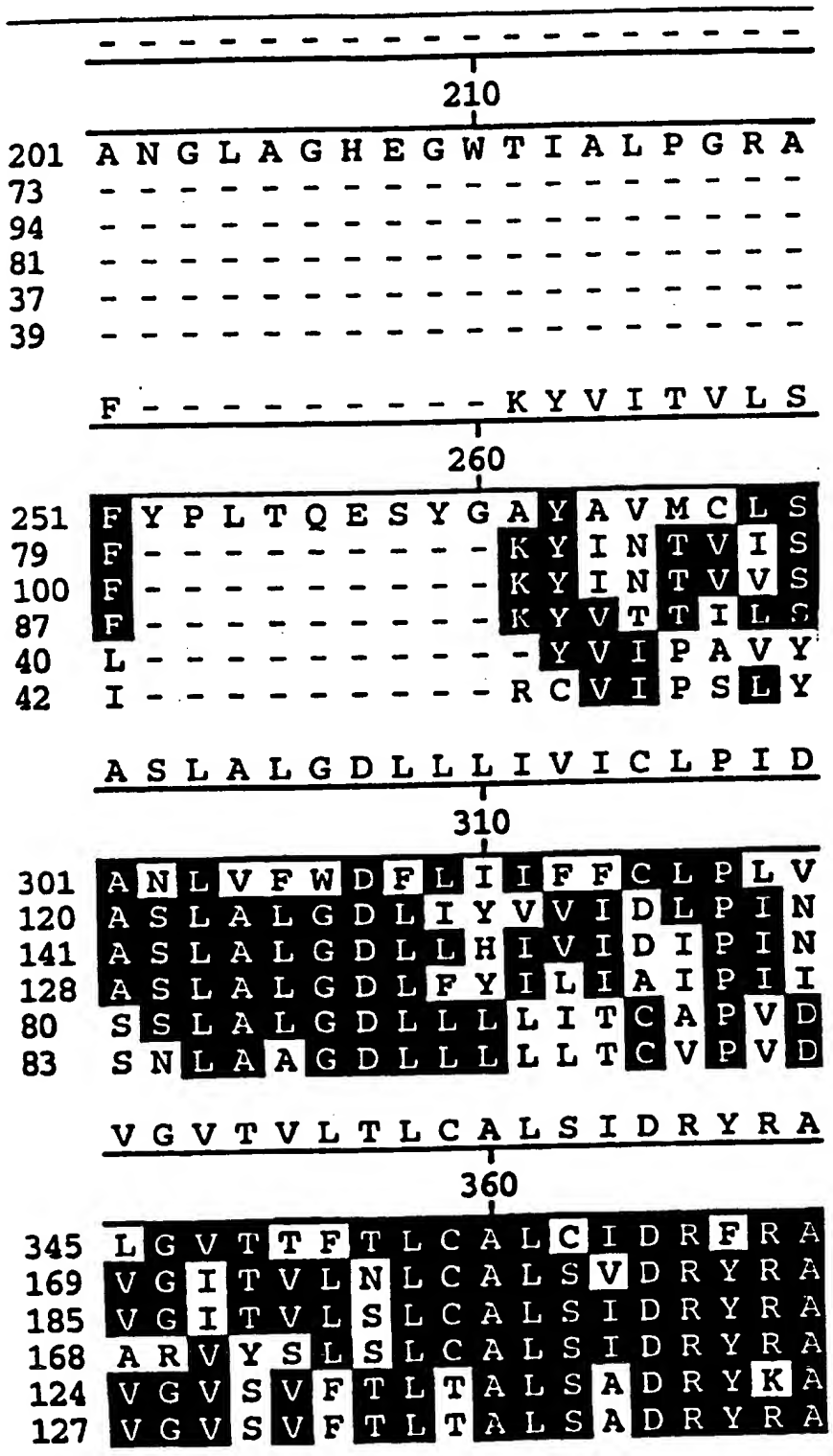
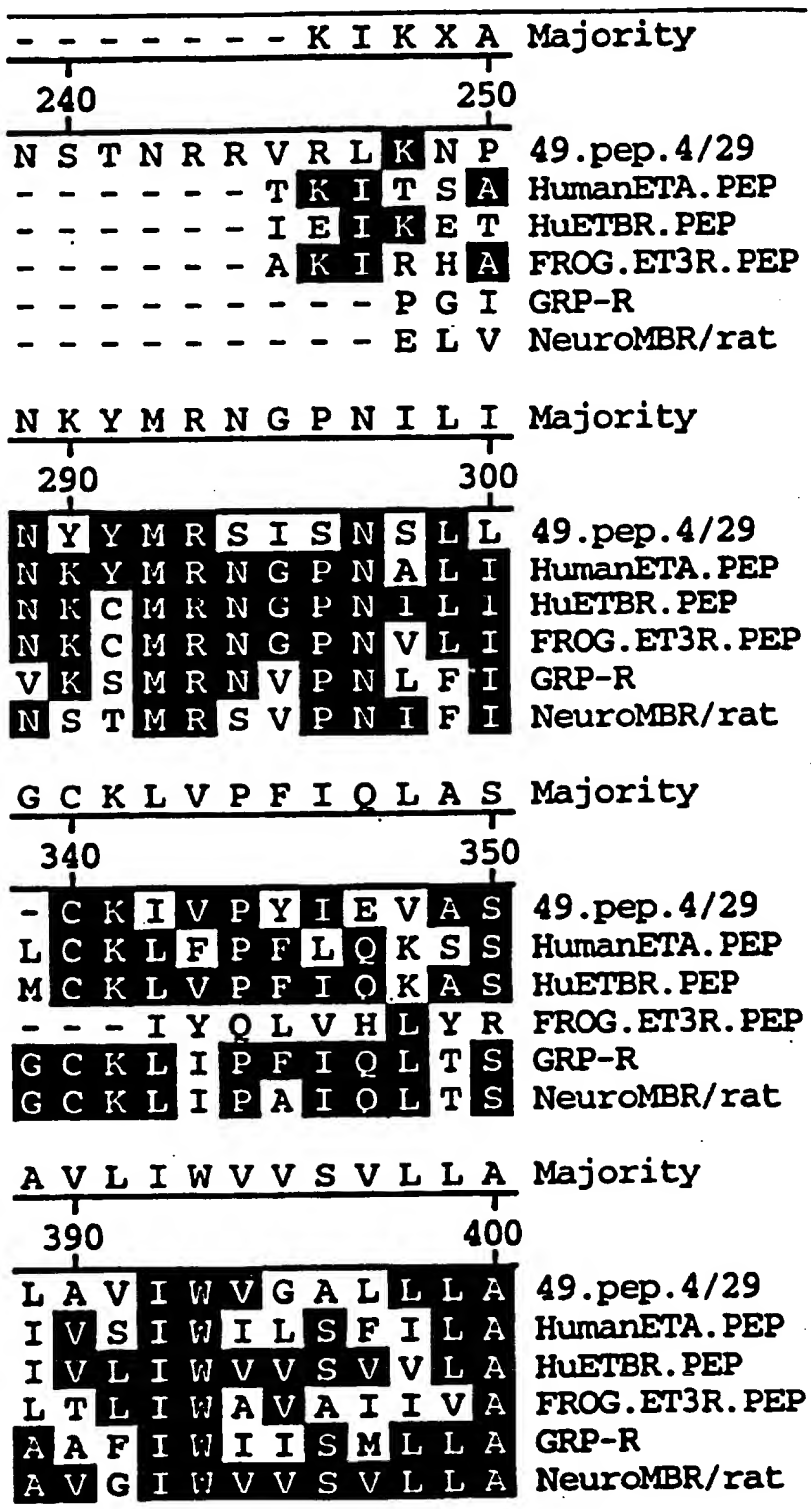


FIG. 3Bb

MATCH WITH FIG. 3Ba

MATCH WITH FIG. 3Bc



16 / 21

FIG. 3Bc

MATCH WITH FIG. 3Bb

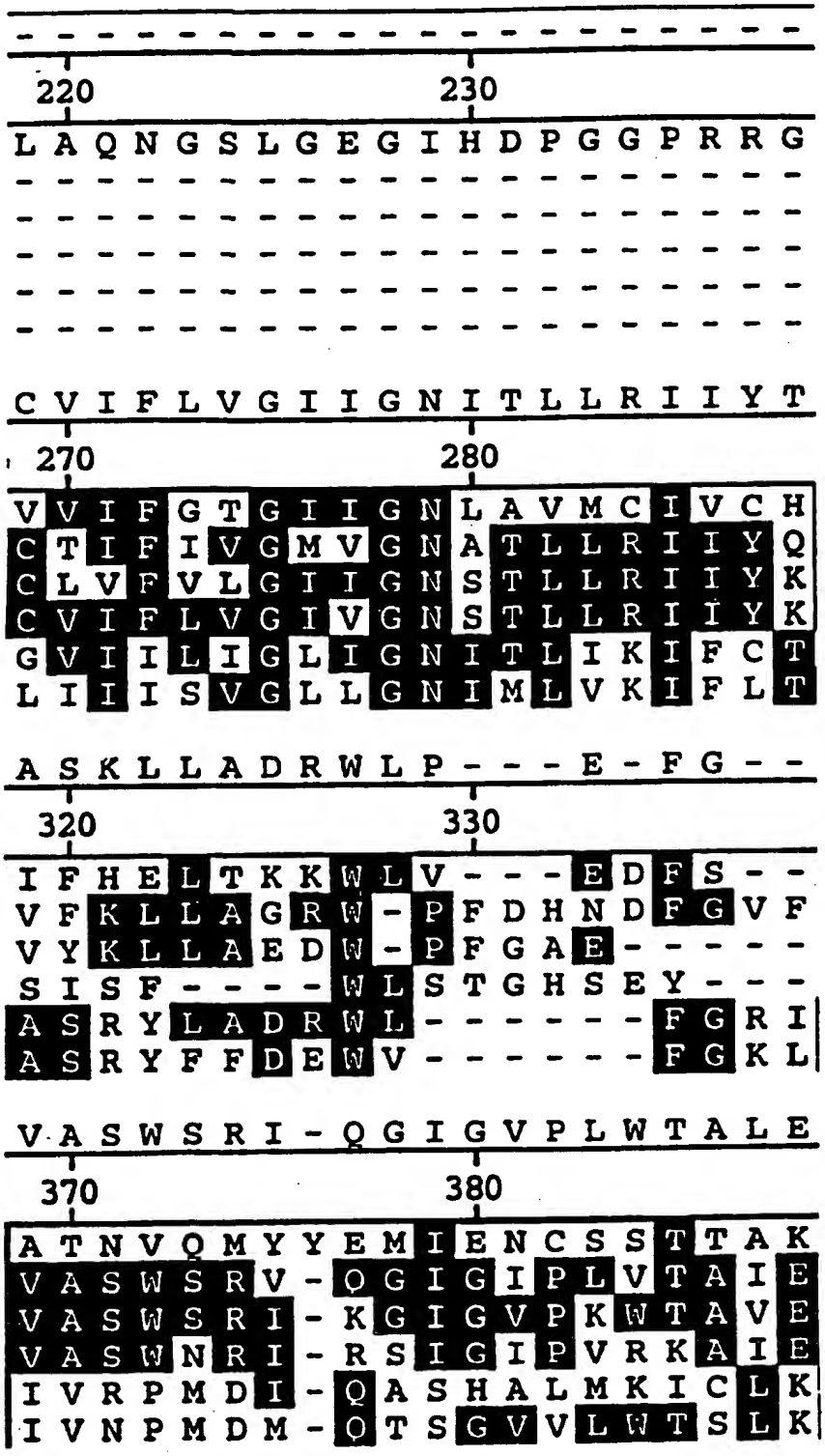


FIG. 3Ca

MATCH WITH FIG. 3Cb

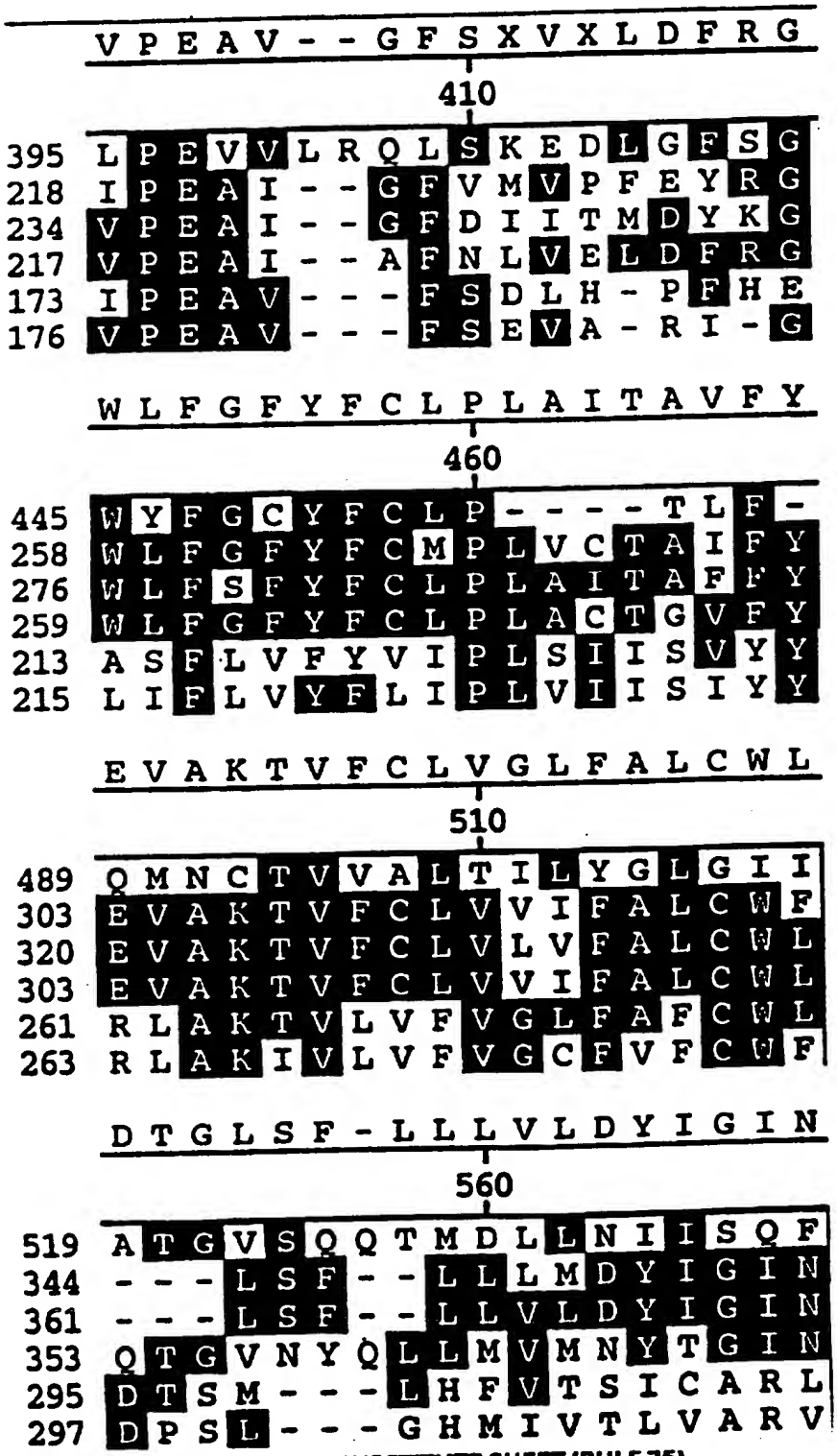


FIG. 3Cb

MATCH WITH FIG. 3Ca

MATCH WITH FIG. 3Cc

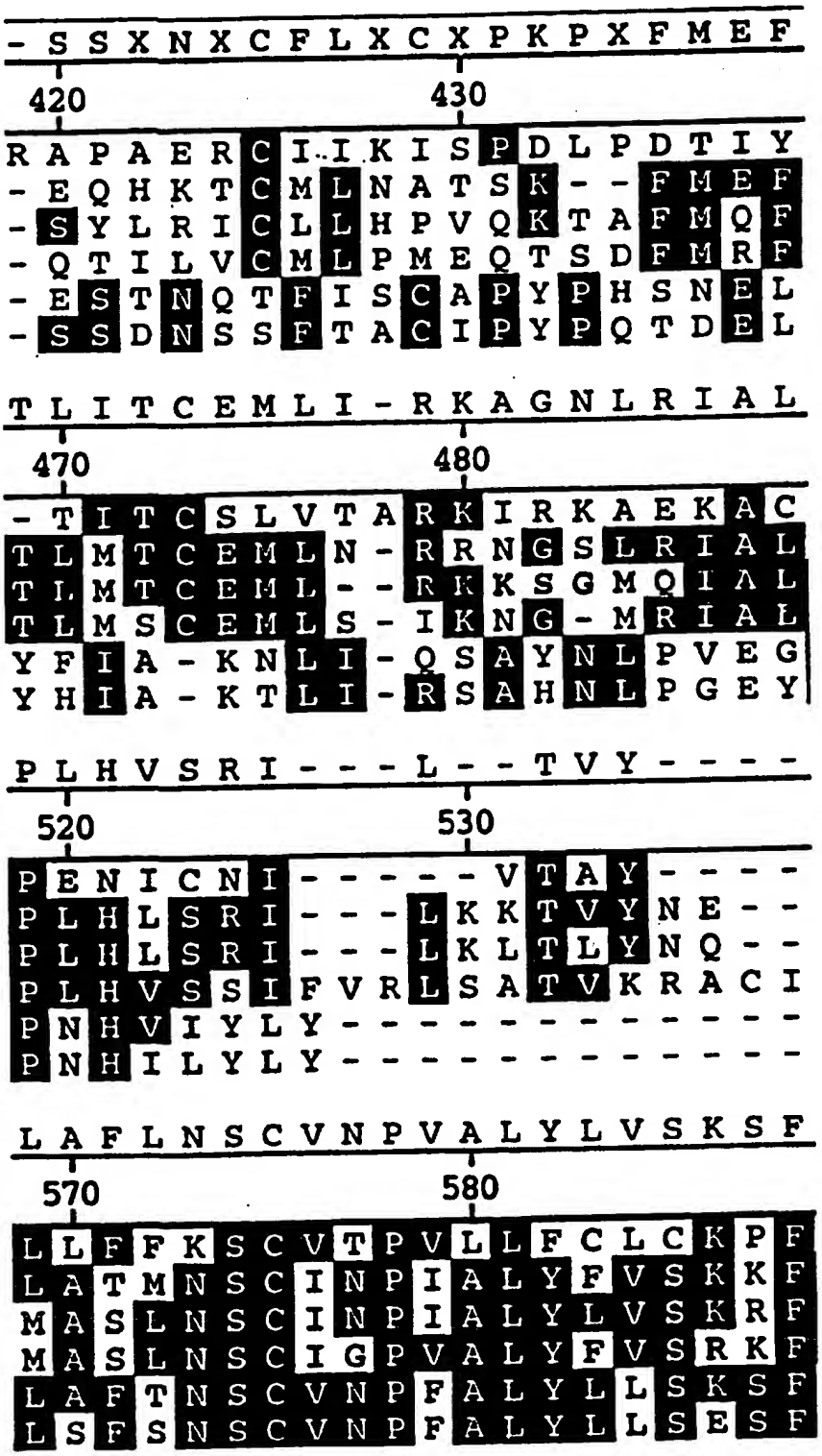


FIG. 3Cc

MATCH WITH FIG. 3Cb

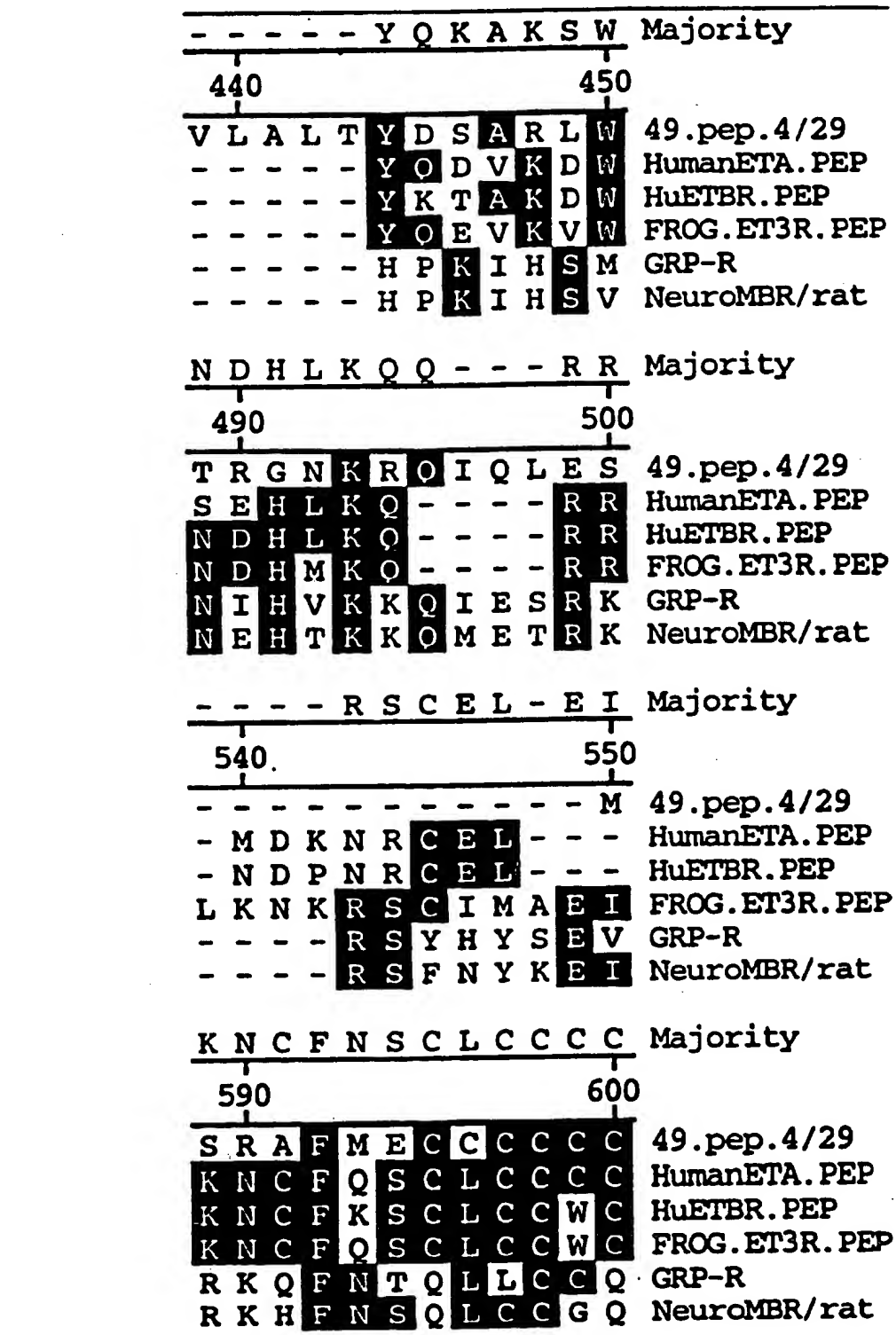
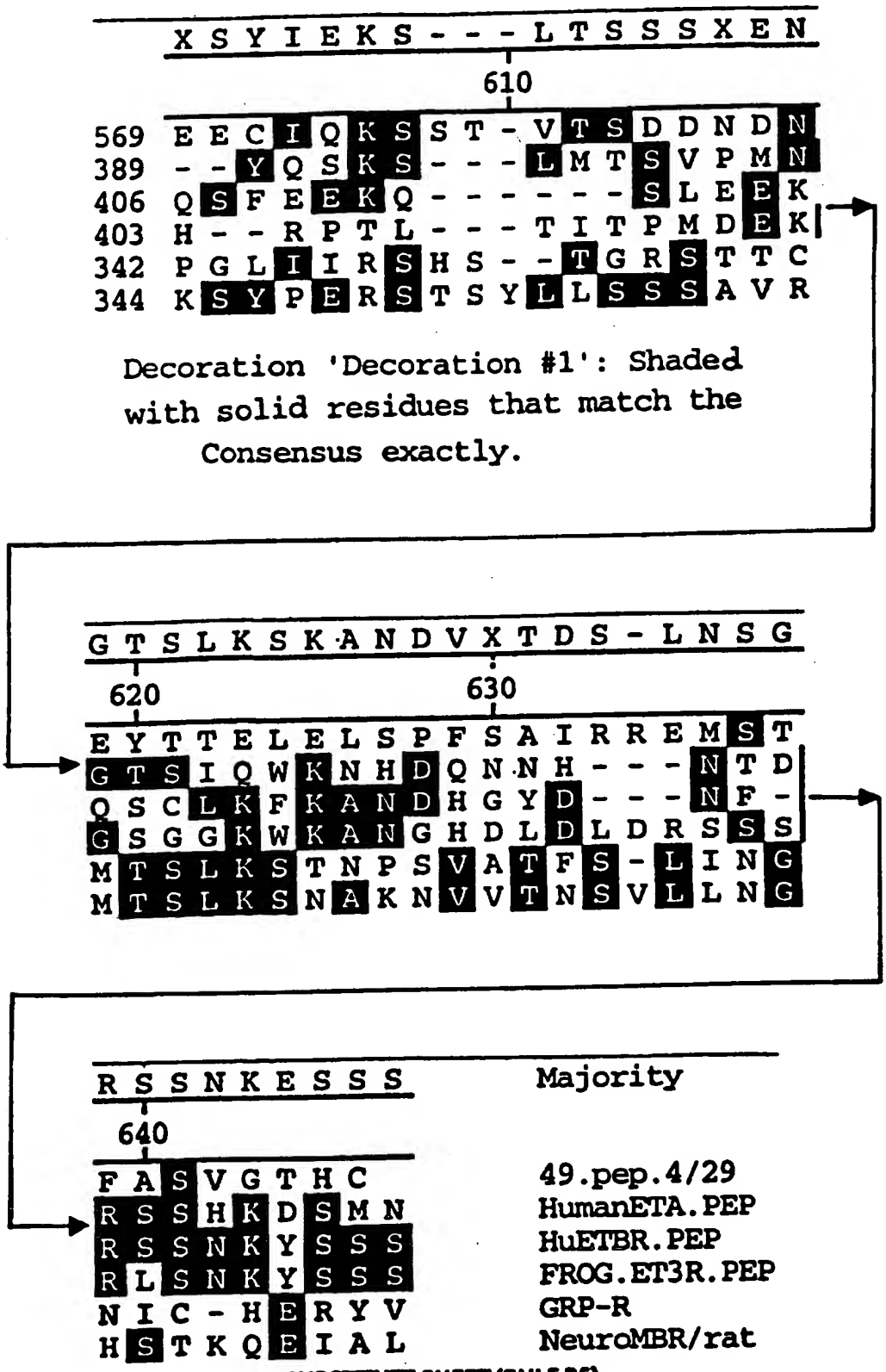


FIG. 3D


X S Y I E K S - - - L T S S S X E N
610
569 E E C I Q K S S T - V T S D D N D N
389 - - Y Q S K S - - - L M T S V P M N
406 Q S F E E K Q - - - - - - - S L E E K
403 H - - R P T L - - - T I T P M D E K
342 P G L I I R S H S - - T G R S T T C
344 K S Y P E R S T S Y L L S S S A V R
Decoration 'Decoration #1': Shaded with solid residues that match the Consensus exactly.
G T S L K S K A N D V X T D S - L N S G
620
630
E Y T T E L E L S P F S A I R R E M S T
G T S I Q W K N H D Q N N H - - - N T D
Q S C L K F K A N D H G Y D - - - N F -
G S G G K W K A N G H D L D L D R S S S
M T S L K S T N P S V A T F S - L I N G
M T S L K S N A K N V V T N S V L L N G
R S S N K E S S S
Majority
640
F A S V G T H C
49.pep.4/29
R S S H K D S M N
HumanETA.PEP
R S S N K Y S S S
HuETBR.PEP
R L S N K Y S S S
FROG.ET3R.PEP
N I C - H E R Y V
GRP-R
H S T K Q E I A L
NeuroMBR/rat

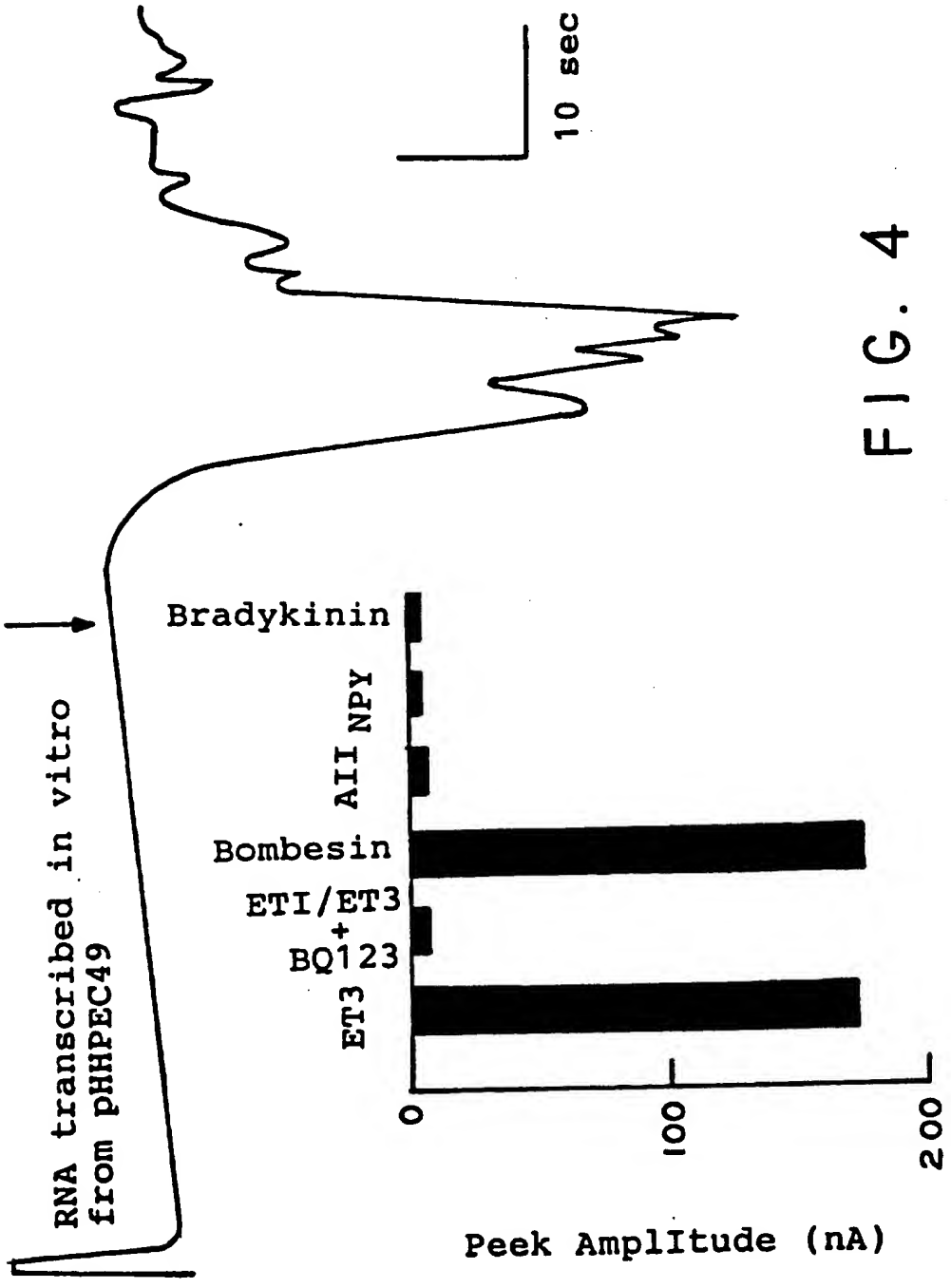

10 sec
ETI(10^{-8}M)
RNA transcribed in vitro from pHHPEC49
Bradykinin
AII
NPY
Bombesin
ETI/ET3
BQ123+
ET3
0
100
200
Peek Amplitude (nA)

FIG. 4

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/11843

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :Please See Extra Sheet.
US CL :435/6, 69.1, 240.1, 320.1; 514/12; 530/350, 388.1; 536/23.1
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/6, 69.1, 240.1, 320.1; 514/12; 530/350, 388.1; 536/23.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, Dialog, Medline, WPI
search terms: endothelin receptor, bombesin receptor, endothelin bombesin receptor

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Biochemical and Biophysical Research Communications, Volume 180, Number 3, issued 14 December 1991, Adachi et al, "Cloning and Characterization of cDNA Encoding Human A-Type Endothelin Receptor", pages 1265-1272, see pages 1267-1270.	1-25
Y	The American Journal of the Medical Sciences, Volume 304, Number 4, issued October 1992, Hayzer et al, "Cloning and Expression of a Human Endothelin Receptor: Subtype A", pages 231-238, see pages 233-238.	1-25

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
23 JANUARY 1995	FEB 03 - 1995
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer Sally P. Teng Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/11843

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Federation of European biochemical Societies, Volume 287, Number 1, 2, issued August 1991, Hosoda et al, "Cloning and Expression of Human Endothelin-1 Receptor cDNA", pages 23-26, see pages 24 and 25.	1-25
Y	The Journal of Biological Chemistry, Volume 262, Number 9, issued 25 March 1987, Zachary et al, "Identification of a Receptor for Peptides of the Bombesin Family in Swiss 3T3 Cells by Affinity Cross-Linking", pages 3947-3950, see pages 3948 and 3949.	1-25

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/11843

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (6):

C07K 14/00, 14/705, 16/00; C12N 15/00; A61K 38/17